THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

VOL. CI—NO. 4

Issued September 1, 1932

BALTIMORE, U. S. A.

1932

Entered as second-class matter, August 18, 1914, at the Post Office in Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918.

Made in United States of America

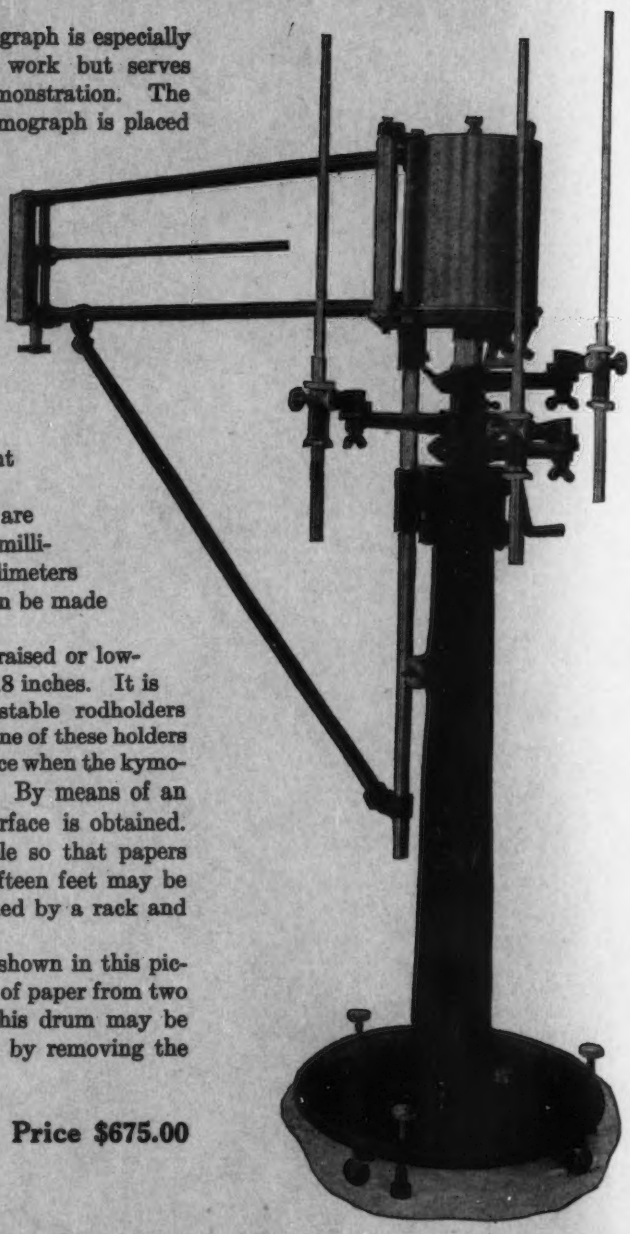
LONG PAPER KYMOGRAPH

THIS forty speed Kymograph is especially designed for research work but serves admirably for student demonstration. The motor which drives the kymograph is placed within the large drum, thus doing away with all troublesome belts and making it possible to mount the complete kymograph on a pedestal, the base of which is 20 inches in diameter and is equipped with castors and leveling screws so that the apparatus may be moved to any convenient place.

Forty changes of speed are available, ranging from 50 millimeters per hour to 110 millimeters per second. All changes can be made rapidly.

The kymograph may be raised or lowered through a distance of 18 inches. It is equipped with three adjustable rodholders and fine adjusting screw. One of these holders moves with the writing surface when the kymograph is raised or lowered. By means of an idle drum a flat writing surface is obtained. The rear drum is adjustable so that papers varying between eight to fifteen feet may be used. The paper is tightened by a rack and pinion.

An additional drum, not shown in this picture, is provided for the use of paper from two to three feet in length. This drum may be adjusted on the upper arm by removing the adjustable rodholder.



Price $675.00

JOSEPH BECKER

630 West 168th Street, **NEW YORK CITY**

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 101 SEPTEMBER 1, 1932 No. 4

THE PREVENTION OF HYPERTROPHY AND THE LIMITATION OF NORMAL PULSATION AND EXPANSION OF THE KIDNEY BY MEANS OF CASTS[1]

SAMUEL SOSKIN AND OTTO SAPHIR

From the Departments of Physiology and Pathology of the Nelson Morris Institute, Michael Reese Hospital, Chicago; aided by a grant from the Max Pam Fund and the John D. Hertz Fund

Received for publication April 23, 1932

A parenchymatous degeneration (cloudy swelling) of the kidney, liver and myocardium is commonly found at postmortem examination in cases of acute infectious disease. The organs in this condition are much larger than normal, and the parenchyma expands and protrudes when the capsule is incised. The harmful effects of this state of affairs as regards the kidney have been clinically recognized. It is generally accepted that such a kidney, within the intact and relatively inelastic kidney capsule, must have existed under an increased internal pressure, and that this pressure must have limited the blood flow through the organ. Surgical incision of the kidney capsule has been resorted to in an attempt to remedy this condition. It has been customary to assume that the cloudy swelling is responsible for the increased size of the organ and hence for the increased internal pressure, although this belief is entirely based on *post hoc* reasoning. It is quite possible, however, that with the development of an increased pressure within the kidney, with or without the presence of some cloudy swelling, this pressure in itself might not only further incapacitate the kidney but might also be responsible for part of the histological picture observed. In other words, the increased internal pressure may exert a deleterious effect upon the kidney and thus establish a vicious cycle.

In this work we have attempted to study the effects of experimental limitation of increase in kidney volume, and the effects of experimentally produced increased pressure within the normal kidney, without primary kidney damage. The limitation of normal pulsation of the kidney, and of

[1] Presented in preliminary form before the American Physiological Society, April 1931, and the Central Society for Clinical Research, November 1931.

the increase in volume which occurs in this organ with active secretion, was accomplished by surrounding the kidney, in a resting state, with a rigid cast. By removing the opposite kidney, we were able to utilize this well known method of stimulating kidney hypertrophy, to cause an increased pressure within the normal kidney surrounded by the cast. We were thus able to observe the effects of prevention of hypertrophy in a normal kidney. The increased pressure within the cast caused by the attempted hypertrophy must, of course, have produced a further limitation to the normal pulsation and expansion of the kidney.

METHODS. Using an aseptic technic, one or both kidneys of a series of dogs were enclosed in rigid casts applied in the form of narrow gauze bandage soaked in collodium and allowed to harden in situ. In several animals, where the cast was applied to one kidney, the opposite kidney

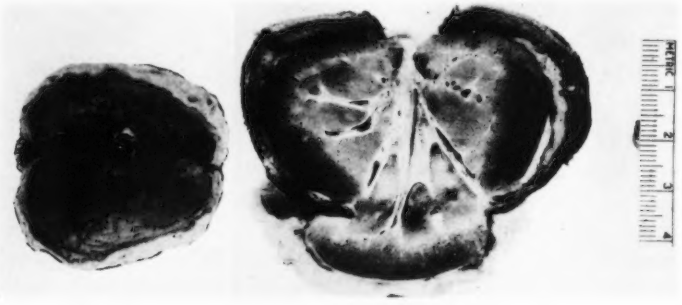


Fig. 1 Fig. 2

Fig. 1. Cast (opened). Note the space for blood vessels and ureter.

Fig. 2. Kidney bulging through window in cast (other kidney previously removed).

was removed at the same or a subsequent operation, in order to produce an attempt at hypertrophy in the enclosed kidney and hence an increased internal pressure. To determine whether the trauma of the operation or the presence of the foreign material composing the cast were harmful to the kidney, control experiments were performed in which the cast or casts were split as soon as they hardened, but were allowed to remain in situ. Some short-term experiments were undertaken to determine the immediate effects of placing a cast about the kidney upon its urinary output.

In order to establish beyond doubt that the removal of one kidney does cause an increased pressure within the remaining kidney enclosed by a cast, the following procedure was adopted. A cast was applied to a kidney and, as soon as the collodium hardened, a round window, approximately 1.5 cm. in diameter was cut through the cast along its greater

convexity. The opposite kidney was removed at the same operation and the abdomen closed.

In all our experiments, short-term and survival, special care was taken to see that the cast did not interfere with the structures at the kidney hilus. To control the immediate effects of trauma and cooling upon the casted kidney in the short-term experiments, the opposite kidney was always subjected to similar manipulative procedures.

All experiments were concluded by histological examination of the kidneys. Blocks of the kidneys were hardened in 10 per cent formalin and imbedded in paraffin. The sections were stained with hematoxylin-eosin. In all instances, frozen sections from different parts of the kidneys were cut and stained with Sudan III for the presence of fat.

TABLE 1

Survival experiments

SERIES	NUMBER OF DOGS	PROCEDURE	PERIOD OF SURVIVAL
A	3	Cast applied to one kidney; opposite kidney removed at same operation	2–3 days
B	5	Casts applied to both kidneys at same operation	1–22 days
C	5	Cast applied to one kidney; opposite kidney removed 1–2 weeks later	1–14 days
D	1	Cast applied to one kidney. Two months later, cast removed and opposite kidney excised at same operation	5 days
E	13	Cast applied to one kidney. Cast removed at a subsequent operation, 1–5 weeks later. Opposite kidney removed at third operation	5 died (4–30 days). 8 survived "indefinitely"

RESULTS. The results are summarized in the following tables which are self-explanatory. The data presented below are taken from those animals which readily recovered from the operative trauma and which presented no complicating factors, such as infections, etc.

Survival experiments. It will be noted that eight animals in series E, table 1, survived for a length of time which might be termed "indefinite."

The procedure in series D was similar to that in series E except that the opposite kidney was excised at the same instead of a subsequent operation to the removal of the cast. The speedy death of this animal suggested that some interval was necessary for recuperation of the liberated kidney. All subsequent experiments were therefore conducted as in series E.

The procedure applied to dogs of series E, table 1, yielded results (summarized in table 2) which may be divided into three distinct groups:

I. One animal died very quickly with nitrogen retention similar to that shown by the animal with both kidneys enclosed in casts.

II. Four animals died after a relatively short period showing little if any nitrogen retention. It will be noted that in two animals the non-protein nitrogen of the blood was higher at an intermediate period than at the time of death. The albumin-globulin ratio of the blood serum was inverted in one dog. The manner of death of these animals will be discussed later.

TABLE 2

*Survival experiments**

Showing detailed observations on dogs in series E, table 1. (Cast applied to one kidney. Cast removed at a subsequent operation 1–5 weeks later. Opposite kidney removed at third operation.)

GROUP	DOG	SURVIVAL PERIOD	BLOOD CHEMISTRY				REMARKS
			Non-protein nitrogen	pH	CO_2	Total protein	
I	SSAH†	8 days	140		46.1	23.2	Casts on both kidneys
	SSA1	4 days	130	7.42	34.8	20.5	
II	SSD	18 days	62	7.65	61.1	13.2	Non-protein nitrogen 256 on 6th day
	SSN	30 days	66	7.43	20.1	16.5	Non-protein nitrogen 92 on 15th day
	SSP	20 days	37	7.70	52.3		
	SSAE	8 days	76	7.40	58.8	15.1	Albumin-globulin ratio = 2.96/1.69
III	SSF	Living (429 days)	30	7.57	42.1	17.9	Albumin-globulin ratio = 2.28/3.40
	SSAA	Living (230 days)	37	7.50	53.8	16.4	Albumin-globulin ratio = 2.53/5.28
	SSAB	Living (230 days)	31	7.50	48.2	19.3	Albumin-globulin ratio = 3.62/2.79
	SSAD	Living (82 days)	36	7.48	50.6	20.6	
	SSA2	Living (70 days)	71	7.48	43.7	15.7	
	SSA6	Living (82 days)	32	7.43	44.3	17.9	
	SSA7	Living (77 days)	20			17.0	
	SSA8	Living (77 days)	34	7.43	38.0	15.4	

* The chemical data presented represent determinations made within the last 48 hours of life in those animals which died, and within a week from date at which time this table was compiled (January 20, 1932) for those animals still living.

† For purposes of comparison, we include SSAH, an animal with casts applied simultaneously to both kidneys.

III. Eight animals survived indefinitely, showing, in general, normal non-protein nitrogen levels and little disturbance in the normal level of other chemical constituents of the blood. It will be noted, however, that dog SSAB shows an inverted albumin-globulin ratio.

Control experiments. The dogs in which the kidneys were surrounded by casts, the casts, however, split as soon as they hardened and allowed to remain in situ, survived indefinitely. This indicates that neither the trauma of the operation nor the presence of foreign material composing the cast is responsible for the results recorded in table 1. Since that portion of the cast which surrounded the kidney hilus was left intact in all of these control animals, it is conclusively shown that our results cannot be due to the encroachment of the cast upon the structures at the hilus. Also, the dog whose one kidney was surrounded by a cast through which a window was cut and whose opposite kidney was removed at the same operation survived for one month, at which time it was sacrificed. At post-mortem examination there was a marked bulging of the kidney parenchyma through the window in the cast, and also a marked eversion of that portion of the cast forming the edges of the window (fig. 2). It is worth mentioning that a similar result was obtained when a cast with a window was applied to a kidney after it had reached a state of considerable hypertrophy following the removal of the opposite kidney. This finding suggests that the attempt at hypertrophy may not be the only change which plays a part in causing the expansion of the kidney through the opening of the cast. The extrusion of kidney substance through the window in the cast and the eversion of the edges of the window show, beyond doubt, that the kidney within the cast must have developed a considerable amount of pressure.

Autopsy findings. In all animals which died with an intact cast enclosing the one remaining kidney, the peritoneal cavity contained a varying amount of a clear or slightly blood stained liquid. Repeated smears from this liquid showed no bacteria. There were marked adhesions between the outer portions of the cast on the kidney and the surrounding structures. Before the casts were removed, the hilus of the kidney was examined in every instance for patency of the arteries, veins and ureters. In no instance was an obstruction of these structures found. The gross and histologic findings in the kidneys were as follows: The organs which had been contained in casts for about two weeks were of normal size and shape, the capsules markedly thickened. The histological examination revealed a varying degree of cloudy swelling and fatty degeneration of the lining cells of the tubuli. In some instances, actual necrosis of these cells was found. These changes were more marked in the tubuli close to the glomeruli. The glomeruli themselves were normal. The kidneys which had been in casts for four weeks or longer were much smaller than normal; they were irregu-

lar in shape due to several bulging areas which were of the same consistency as the neighboring kidney tissue. The histologic examination showed cloudy swelling of the lining cells of the tubuli. Necrosis was much less frequently observed than in the kidneys described before. There was much connective tissue extending from the capsule into the cortex; but no inflammatory changes were found within the glomeruli. The kidneys which had been enclosed in casts for some time, the casts, however, having been removed prior to the death of the animals, showed microscopically a marked fibrosis of the capsule, which extended into the surrounding cortical tissue. There was a moderate infiltration of lymphocytes throughout the cortex. Occasionally, fat droplets were found in the lining cells of the

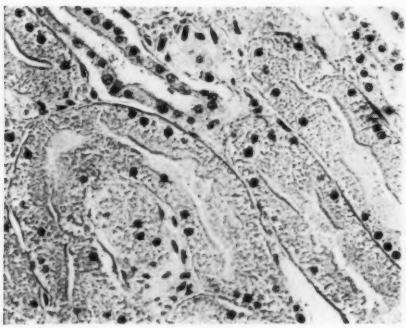


Fig. 3

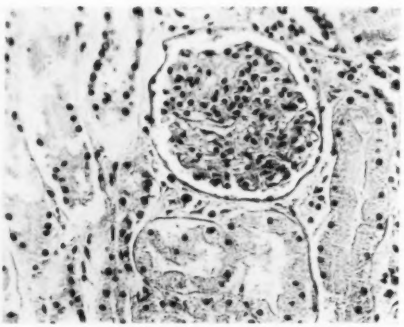


Fig. 4

Fig. 3. Marked cloudy swelling and early necrosis of the tubular epithelium (from a kidney which was left in a cast for a period of two weeks). Hematoxylin-eosin preparation. ×260.

Fig. 4. Cloudy swelling of the tubular epithelium, and normal glomerulus (from a kidney which remained in a cast for one week). Hematoxylin-eosin preparation. ×180.

tubuli close to the glomeruli. The glomeruli and many tubular lining cells appeared normal. The kidney enclosed in a cast through which a window had been cut showed a distinct bulging of the kidney structure through the window. On section, it was noted that the portion of kidney which projected through the window measured 1.3 cm. in diameter (fig. 2). On histologic examination, there was a slight lymphocytic infiltration throughout the cortex, and some connective tissue proliferation. An occasional glomerulus showed a slight irregularity of its loops, and a few glomeruli contained a reddish granular material in the capsular space. A moderate cloudy swelling of the lining cells of the tubuli was noted. Some of them also contained a few fat globules. Histologic examination of the kidneys

TABLE 3

In this animal, the left kidney was placed in the cast three days before the acute experiment. At the time of the experiment, it may be seen that the encasted kidney was excreting approximately 3 per cent of the amount of urine excreted by the opposite normal kidney. This small amount of urine was very concentrated as to its sugar and nitrogen content. After the cast was removed, the left kidney excreted increasing volumes of urine as compared to the opposite kidney and the urine became proportionately less concentrated. At the end of the experiment, the left kidney was excreting approximately 50 per cent as much urine as the undisturbed kidney.

TIME	MANIPULATION	BLOOD PRESSURE	NORMAL KIDNEY (RIGHT)					CASTED KIDNEY (LEFT)				
				Sugar		Nitrogen			Sugar		Nitrogen	
			Volume	Total milligrams	Milligrams per cc. urine	Total milligrams	Milligrams per cc. urine	Volume	Total milligrams	Milligrams per cc. urine	Total milligrams	Milligrams per cc. urine
		mm. Hg	cc.					cc.				
3–23–31	Cast applied to left kidney											
3–26–31 4:35	Abdomen opened and ureters catheterized under amytal anesthesia	132										
4:47	50 cc. 10 per cent glucose intravenously	144										
4:49–5:04	Urine collected		47.2	286	6.1	38	0.8	1.5	41	27.3	22	14.7
5:10	Cast removed from left kidney	119										
5:21–5:36	Urine collected		17.65	202	11.5	30	1.7	6.4	157	24.5	25	3.9
5:41	50 cc. 10 per cent glucose intravenously	106										
5:42–5:57	Urine collected	88	10.4	215	20.7	28	2.7	5.1	155	30.4	21	4.1
6:02	0.5 gm. caffeine sodium benzoate intravenously	91										
6:04–6:19	Urine collected		6.3	7	1.1	30	4.8	2.7	6	2.2	24	8.9
6:33	50 cc. 10 per cent glucose intravenously. 50 cc. physiological saline intravenously	103										
6:35–6:51	Urine collected		8.4	102	12.1	30	3.6	4.0	94	23.5	19	4.8

removed from the control animals in which the casts had been split as soon as applied, but allowed to remain in situ, showed a slight granularity of the cytoplasm of the lining cells of the tubuli in some fields; but many

tubuli appeared normal. No fat could be demonstrated, and there was no evidence of necrosis.

Short-term experiments. In a series of ten dogs, short-term experiments were undertaken to determine the immediate effects of the application and removal of casts on the secretory activity of the kidney. A cast was applied to one kidney of an animal either immediately before or several days before the experiment. The normal kidney was used as a control in each case. Under amytal anesthesia, catheters were inserted into both ureters and the urine excreted by each kidney collected separately and compared as to volume and composition. Table 3 illustrates such an experiment.

Discussion. When a cast was placed on one kidney of a dog and the opposite kidney removed, the animal invariably died. Death resulted whether the nephrectomy was performed at the same or a subsequent operation to the placing of the cast. A similar result was always obtained when both kidneys were simultaneously enclosed in casts. There can therefore be no doubt that the presence of the cast severely impaired the functional activity of the kidney. This is also indicated by the observation that when one kidney had been in a cast for some time, the opposite kidney was found to have hypertrophied. When, however, a cast was applied to one kidney, the cast split as soon as it hardened but allowed to remain in place, and the opposite kidney then removed, the animal survived. Similarly, when casts were applied to both kidneys, and the casts were split as soon as they hardened but allowed to remain in place, the animal survived. Neither the trauma of the operation nor the presence of foreign material around the kidney could therefore have caused significant damage. These control animals also prove that the impairment of kidney function in our experiments was not the result of interference with the patency of the blood vessels or ureter at the kidney hilus.

The survival of the animal whose one remaining kidney was enclosed in a cast through which a window had been cut is very significant. Like the other control experiments, it shows that neither the trauma of the operation, the presence of the foreign material about the kidney nor the interference at the kidney hilus can account for the death of those animals whose kidney or kidneys were surrounded by intact casts. The extrusion of kidney substance through an aperture only 1.5 cm. in diameter, and the eversion of the rigid material around the edges of this opening show, beyond doubt, that the kidney within the cast must have developed a considerable amount of pressure.

When a cast was applied to one kidney, the cast removed two to four weeks later, and the opposite kidney removed at a third operation, 8 out of 13 animals survived. This further indicated that the application of a cast might leave no severe or permanent kidney damage, after the cast

was removed. The death of the animals must therefore be attributed to the volume limitation imposed by the intact cast upon the kidney or kidneys carrying on the renal activity essential to the life of the animal.

The animals which died with their kidney or kidneys enclosed in casts showed a progressively increasing nitrogen retention up to the time of death. Of the animals which were observed following the removal of the cast from their one remaining kidney, only one (group I, table 2) showed such nitrogen retention. The other four animals which did not survive (group II, table 2) showed no significant nitrogen retention. The death of these latter animals, due without doubt to kidney dysfunction, but not associated with nitrogen retention, forms an interesting subject for further study. These animals died in convulsions and in one case where the brain was carefully examined, a marked edema and hyperemia were found. This suggests the clinical diagnosis of so-called "anuremic uremia."

In the short-term experiments, the placing of a cast about one kidney was immediately followed by a marked reduction of its urinary output as compared with that of the opposite free kidney. This occurred even when both kidneys were denervated prior to the experiment, so that nervous inhibition could not have been responsible for the decreased secretory activity of the encased kidney. A markedly reduced urinary output was also observed to occur in kidneys enclosed in casts several days prior to the short-term experiments. However, as soon as the casts were removed from such kidneys, they began to excrete increasing amounts of urine, which soon approached the output of the opposite undisturbed kidneys. The highly concentrated character of the urine excreted by the kidneys in casts or shortly after liberation from such casts is difficult to explain. In view of the fact that the kidney tubules showed the chief histological changes, while the glomeruli were apparently normal, the secretion of a concentrated urine as compared with that secreted by the undisturbed kidney of the opposite side seems difficult to reconcile with the modern reabsorption theory of urinary secretion. Further experiments in this regard are now in progress.

These short-term experiments demonstrate the impairment of kidney function by limitation of expansion, even in the absence of the stimulus to hypertrophy or greatly increased internal pressure. They also show the rapid resumption of function once the cast is removed. It is not difficult to imagine that a similar condition might arise through the restraining influence of the kidney capsule upon the expansion of kidney parenchyma which may be undergoing a sudden cloudy swelling. Once the increased internal pressure is present, the vicious cycle is set in motion leading to more cloudy swelling and a greater increased internal pressure, etc. These acute experiments suggest a simple mechanical explanation for the occurrence of some cases of temporary oliguria or anuria. Since this explanation

does not depend upon the assumption of a severe pathological process in the kidney, it seems especially applicable to those instances in which the kidney dysfunction disappears as rapidly as it appeared, leaving no evidences of marked kidney damage. This might be accounted for by the breaking of the vicious cycle, either through the stretching of the kidney capsule with time, or the alleviation of the original cause of the initial cloudy swelling.

SUMMARY

In a series of dogs, kidneys were aseptically enclosed in gauze and collodium casts, care being taken to avoid interference with the blood vessels and ureter at the hilus. When one kidney of an animal was so treated and the other kidney removed, at the same or a subsequent operation, the animal invariably died. If, however, the cast was removed from the one kidney before the other kidney was extirpated, the animal often survived. Various control experiments indicated that the procedure employed caused an increased internal pressure within the kidney enclosed by the cast, and that neither the trauma of the operation nor the presence of the foreign material about the kidney appreciably influenced the results obtained.

These experiments show that a significant impairment of functional activity occurs in kidneys surrounded by a rigid cast. This impairment does not depend on extensive or permanent kidney damage. These results therefore indicate that the procedure which we have employed interfered with normal physiologic conditions necessary to kidney function. Such conditions may be:

1. The normal pulsation coincident with the pulse pressure
2. The normal expansion during active secretion
3. The hypertrophy stimulated by removing the opposite kidney or incapacitating it with a cast.

Although the prevention of hypertrophy may have played a rôle in the survival experiments, the short-term experiments indicate that the interference with normal pulsation and expansion of the kidney was probably the chief factor involved. The increased internal pressure resulting from the attempt at hypertrophy within the rigid cast must have increased the limitations imposed by the cast. It is not improbable that similar conditions are responsible for some of the functional impairment and pathological changes in kidneys which are ordinarily considered to be manifesting the results of a simple parenchymatous degeneration. The appearance of this cloudy swelling in normal kidneys, as a result of the conditions set up by our procedure, is significant.

THE PHYSIOLOGICAL ACTIVITY OF IODINE IN THYROGLOBULIN

BRODA OTTO BARNES

From the Department of Physiology, University of Chicago

Received for publication May 18, 1932

Attempts to prepare a characteristic substance from the thyroid gland began certainly as early as 1884 when Bubnow (1) prepared "Thyreoprotein." Langerdorff (2) made some observations on the colloid of the thyroid and noticed that it became readily soluble when subjected to artificial gastric digestion. Gourley (3) prepared a substance which he called the "principal proteid" of the thyroid. From the properties of his compound, it must have been the same as Bubnow's preparation. It remained for Baumann (4) to discover iodine in the gland in 1895. His correlation of the iodine content with diseases of the thyroid not only paved the way for iodine therapy but also gave a chemical means of following the active principle in thyroid preparations. Attempts to concentrate or isolate iodine-containing compounds characteristic of thyroid action may be classified under the three following heads: acid hydrolysis, alkaline hydrolysis, and by the use of enzymes. Only the latter will be considered here since it is closer related to experimental hyperthyroidism produced by feeding thyroid preparations.

Most of the work involving enzyme hydrolysis is open to one of two criticisms: either very little was done in attempting to separate the iodine compound or else the fractions were not properly assayed. Hutchison (5) as early as 1896 reported that the protein which Bubnow and Gourley had described contained iodine. He carried out a series of experiments on this compound including the solubility, color reactions, heat of coagulation, hydrolysis with acid, and the effect of gastric digestion. By the latter method, he separated the iodine into two fractions which were then assayed on thyroidectomized dogs in tetany. His conclusions are unwarranted in view of the present conception of tetany and the parathyroids. The same author (6) later reported the separation of digested thyroid into three fractions: iodothyrin, albumoses and peptones. The iodine content decreased in the order mentioned. They were tested on a case of myxedema and their effect on loss of weight was proportional to their iodine content. Tamback (7) studied the effect of both pepsin and trypsin on

thyroid preparations, but did not study their physiological activity. Some digestion studies on human thyroids were carried out by von Cyon and Oswald (8), but their products were tested on the circulation and not on thyroid deficient animals. Oswald (9) in 1908 reported quantitative studies on thyroglobulin digested with trypsin. His report that as much as two-thirds of the iodine might be liberated in inorganic form has probably kept many workers from using enzymes. However, it must be remembered that the active principle is not destroyed, at least entirely, when thyroid is fed. Furthermore, Oswald did not examine his mixtures until months after their preparation. The length of time and possible bacterial contamination might have added to the decomposition. Nurnberg (10) carried out some extensive chemical studies on the products after digestion of thyroglobulin, but he did not test them physiologically. Pick and Pineles (11) were the first to combine both separation and adequate assay. Using thyroidectomized goats which displayed cretin symptoms, they found that fresh thyroids, thyroglobulin, and secondary albumoses relieved the thyroid deficiency. On the other hand, iodothyrin, primary albumoses, and digestive mixtures standing three months were ineffective. Cameron and Carmichael (12) made the first attempts to compare the effects of undigested thyroglobulin with the products of digestion. Although they state that their method of assay, using body weight and hypertrophy of the kidneys, liver and heart, is not quantitative, the digested mixture had the same order of activity as the original protein. They examined the residue after digestion, the filtrate, and, in one case, the peptones prepared from the filtrate. All of these fractions were active by their assay. Last year Harington and Salter (13) employed enzymes for the isolation of thyroxine from thyroglobulin. They discarded the filtrate at pH 5.0, apparently without testing its potency. This is not surprising, since Kendall (14) had reported that the acid-soluble fraction after alkaline hydrolysis was inactive. Harington and Randall (15) had confirmed this and shown that thyroxine was quantitatively precipitated at pH 5.0.

Numerous reports have been made that, in equivalent amounts of iodine, thyroxine is less active than desiccated glands. On the other hand, it seems definitely agreed that only a portion of the iodine in the thyroid is present as thyroxine. Therefore one must postulate either that the active principle is more potent than thyroxine per se or that other compounds are present in desiccated thyroid which add to the effects of thyroxine. A study was undertaken to test various fractions of artifically digested thyroglobulin. Since it was found that the filtrate at pH 5.0, after tryptic digestion, might contain as much as fifty per cent or more of the total iodine, this filtrate was examined in some detail for its physiological actions.

METHODS. 1. *Preparation of thyroglobulin digest.*[1] The thyroglobulin used was prepared by a method to be described later. The principle involved the precipitation of the protein from thyroid extract at the isoelectric point. A portion of the thyroid extract was reserved in order to compare the effects of the original active principle with it after digestion. The precipitated thyroglobulin was put into one per cent sodium bicarbonate, and commercial pancreatin was added to a concentration of 0.2 per cent. A few drops of alcoholic thymol were added as a preservative. Although the bicarbonate tends to keep the reaction of such a mixture slightly alkaline, the pH was adjusted daily to 8.0. The pancreatin was renewed after 48 hours. After being in the incubator for 72 hours, the mixture was brought to a pH of 5.0, and the precipitate was collected on a filter. This procedure differs from Harington's only in the preparation of thyroglobulin and in the presence of the bicarbonate. A control solution was prepared by digesting casein in a similar manner. However, this digest was not fractionated.

2. *Work on rats.* Since the digested filtrate might contain toxic substances, it was administered orally by a stomach tube. This was accomplished under light ether anesthesia. The animals were placed on their backs and a small tube attached to a syringe was gently pushed down through the esophagus into the stomach. The fluid could then be injected without any trouble or danger of loss. The dosage varied from 2 to 4 cc. depending on the size of the animal. The procedure was so simple that the rat was under the anesthetic only 2 or 3 minutes.

3. *Work on rabbits.* Two rabbits were given the filtrate by means of a stomach tube, and two others were given the dried precipitate put into capsules. No anesthesia was necessary. When the capsules were given, the animals were observed closely until they had swallowed all of the material. The body weight and rectal temperature were recorded daily.

4. *Work on dogs.* Three dogs were employed for basal metabolism studies. They were trained to lie quietly, and the calories per 24 hours were determined using the Benedict portable apparatus. After control observations had been made (at least 10), the filtrate was given to two of the dogs by stomach tube. Five doses were administered and the heat production, heart rate, respiration and rectal temperature determined. At the end of this experiment, one of these dogs and the third one were fed the precipitate after digestion of the thyroglobulin. The quantities of both the precipitate and the filtrate employed contained the same amount of iodine.

RESULTS. In a preliminary experiment, two preparations of the filtrate at pH 5.0 were prepared and tested for thyroid activity by the loss of

[1] I am indebted to Armour & Company for the fresh thyroids.

weight in rats. Four animals were given each preparation, and four animals were anesthetized in the same way and given a similar quantity of tap water as a control. Of the 8 experimental animals, all lost weight at the beginning but recovered somewhat at the end of 3 weeks. The weight losses amounted to as much as 20 grams in several cases, and one animal died with a loss of 28 grams. At the end of three weeks only one animal was heavier than at the beginning, while the controls had gained weight consistently. These experiments indicated that there was some thyroid-like activity in the filtrate. It was then decided to use more rats, rabbits, and basal metabolism tests on dogs in order to investigate this indication thoroughly.

Experiments on rats. To examine further the effects of ether on growth and the body weight, forty white rats ranging from 70 to 130 grams in weight were divided into two groups. Half of these were anesthetized daily for a period of 7 days. Neither group showed any weight loss during the week, and at the end of the experiment the controls had gained an average of 15.7 grams while those anesthetized averaged 13 grams. If ether did retard growth, certainly it was to a minor degree. More animals would have to be employed to be sure that there was any significant effect. A few days intervened before the same animals were divided into three groups, two of ten each and one of twenty. One group of ten received the original thyroid extract, the other small group received the same volume of casein digest. The large group was given the same volume of digested thyroglobulin filtrate, which contained the same quantity of iodine as the original thyroid extract. The dosage was regulated so that it contained an amount of iodine equivalent to two grams of U. S. P. desiccated thyroid per kilo body weight. The average daily variation for each group is recorded in figure 1. It can be seen that all three groups lost weight at the beginning, but the effect was much greater with the thyroid extract. No explanation can be given for the loss of weight in the animals on casein, where growth was retarded, since at the end of the week they had gained only 4.7 grams. According to the experiment where only ether was given, the gain should have been 13 grams. Since this weight loss has been obtained once with casein and three times with thyroglobulin fractions which contain very little if any thyroxine, one is forced to raise the question whether toxic substances which cause loss of weight are produced by artificial digestion. No attempt will be made to answer this question at present, but the loss of weight in rats has been abandoned as a method of thyroid assay.

The group of animals on the filtrate from digested thyroglobulin lost a little more weight than those on casein, and at the end of the week were only back to their original weight. One cannot interpret this as an indication of thyroid activity in view of what has just been said. However,

their weight loss was in no way comparable with that of the animals on thyroid extract. Here the weight loss was much more severe and five of the animals had died within four days. On the seventh day the dosage of both the original extract and the digested filtrate was increased. Three days later only two of the ten rats on the extract were alive and only one from the group of 20 had died. This latter animal had lost no weight and probably died from other causes. These results clearly show that the digested filtrate contains very little thyroid activity in comparison to the original extract, when equal quantities of iodine are administered.

Experiments on rabbits. The dosage in terms of iodine for the rabbits receiving the filtrate was equivalent to 2 grams of desiccated thyroid per kilo. Unfortunately one of these animals was killed accidentally early

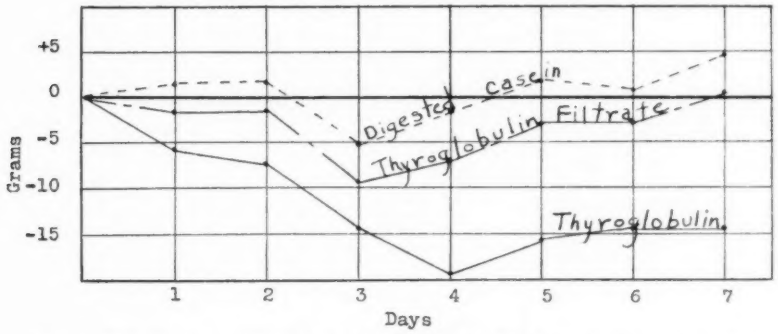


Fig. 1. Showing the average daily changes in weight of rats fed digested casein, undigested thyroglobulin, and the filtrate obtained by bringing digested thyroglobulin to pH 5.0.

in the experiment. The data are summarized in table 1. The rabbit receiving the filtrate continually lost weight during the ten-day period. Although in this case a total of over 400 grams was lost, there was never any diarrhea observed. After the experiment stopped the animal started to regain weight, but observations were not continued until recovery was complete. This experiment furnishes further evidence that there is a factor causing loss of weight, and in this instance there was no anesthesia. There seems a significant difference, however, between the rats and the rabbits. The rats make some adjustment to the toxic factor while the rabbits do not, at least within ten days.

The two rabbits fed the precipitate from thyroglobulin digest exhibited quite a different reaction. Their weight loss was so severe that only three doses were given. The dosage in this case was only three-fourths as great, or the equivalent of one and one-half grams of desiccated thyroid

per kilo. Both animals had severe diarrhea from the second day until they died. When their weights had dropped over 400 and 300 grams respectively in 3 days, the feeding was stopped in order to allow recovery. However, the weather was hot at this time and the animals did not survive the toxicity, one dying the following day and the other 24 hours later. This rapid loss of weight would lead one to believe that the precipitate was very active physiologically, but no quantitative comparisons can be made.

No change in the temperature of the animal receiving the filtrate was ever noticed. Daily fluctuations with changes in the weather were the same as in the control animals. The same may be said for animal 4. The day before animal 3 died his temperature was 2°C. higher than at any other time. In summarizing the data on rabbits, it would appear

TABLE 1

The daily weight of rabbits fed the filtrate and the precipitate obtained by bringing digested thyroglobulin to pH 5.0

DAYS	FILTRATE		PRECIPITATE	
	No. 1	No. 2	No. 3	No. 4
0	2,420	1,740	1,960	1,960
1	2,360	1,700	1,900	1,930
2	2,320	1,620	1,680	1,740
3	2,240	Killed	1,540	1,620
4	2,200		Dead	1,560
5	2,140			Dead
6	2,160			
7	2,120			
8	2,060			
9	2,000			
10	1,960			

that there is some factor in the filtrate which causes loss of weight but does not cause diarrhea or elevation of body temperature. The precipitate causes a much more rapid loss of weight, severe diarrhea, and may elevate body temperature.

Experiments on basal metabolism. Both the filtrate and precipitate were tested for their effect on basal metabolism, since it is probably the final test for thyroid activity. Large doses which were equivalent in iodine to 13 grams of desiccated thyroid were employed. Five daily doses were given to dogs 1 and 2. It can be seen in table 2 that the filtrate had no effect on basal metabolism. This was a surprise since the weight loss in both rats and rabbits had indicated some activity. Just how little hormone is necessary to raise basal metabolism cannot be stated, but from the work of Kunde (16) it would appear that there was not much, if any,

of the active principle present. There was no change in heart rate, temperature, or respiration that would indicate any hyperthyroidism.

When the precipitate having an amount of iodine equivalent to that in the filtrate was fed the basal metabolisms showed a significant rise within 24 hours. Dog 2 showed a gradual rise until on the third day the heat production was over 31 per cent above the controls. Only three doses were fed after which the basal metabolic rate came down gradually. On the seventh day, or 4 days after the last feeding of the thyroid preparation, the basal metabolism was within the control range. Dog 3 did not reach the high peak until the fourth day although none of the material

TABLE 2

The daily changes in basal metabolism and heart rate produced by feeding the filtrate and precipitate obtained by bringing digested thyroglobulin to pH 5.0

DAYS	DOG 1		DOG 2		DOG 3		REMARKS
	Calories per 24 hours	Heart rate	Calories per 24 hours	Heart rate	Calories per 24 hours	Heart rate	
0	341.3	102	330.5	61	439.0	54	Average of controls
1	335.1	105	334.2	62			Given 100 cc. filtrate
2			325.6	60			Given 100 cc. filtrate
3	344.7	93	329.6	60			Given 100 cc. filtrate
4	340.6	80	326.6	62			Given 100 cc. filtrate
5	322.3	78	353.2	60			
0							1.1 gm. pt. fed
1			366.8	70	483.7	74	1.1 gm. pt. fed
2			387.9	84	479.9	64	1.1 gm. pt. fed
3			433.7	84	512.6	73	Fed no thyroid
4			411.3	74	600.5	79	Fed no thyroid
5			389.9	68	504.0	63	Fed no thyroid
6			356.2	62	492.0	58	Fed no thyroid
7			347.2	58			

was fed on the previous date. The return to normal was also slower than the other dog. The heart rate paralleled the basal metabolism in both cases and was surprisingly close in dog 2, whose normal rate was quite constant and never varied over 4 beats per minute in the control period. The heart rate in each case returned to normal before the heat production. In dog 2 the temperature was elevated 0.5°C. on the third, fourth, and fifth days. Dog 3 showed a rise of 0.2 degree only on the fourth day, when his basal metabolism was also at its peak. Respiration did not parallel the heat production as closely as the heart beats, but the respiratory rate on the day of maximum basal metabolism was double the rate at any other time.

Although these observations indicate that the precipitate has considerable potency, no quantitative comparisons can be made. It is apparent that the iodine of the thyroglobulin can be split into two definite fractions by the use of pancreatin. Since one of these fractions shows comparatively little, if any, activity, it would seem conclusive proof that all of the iodine is not associated with the active principle. This agrees with the evidence of Kendall and Harington that all of the iodine is not present as thyroxine. However, the evidence that desiccated thyroid is more active per iodine content than thyroxine has not been satisfactorily explained. Further studies on the precipitate will be made in order to see if some of the active principle might have been destroyed by the artificial digestion.

SUMMARY

1. By the use of pancreatin, thyroglobulin was split into an acid-soluble fraction and an acid-insoluble fraction. The iodine content of each fraction was about the same in some cases. The acid-soluble fraction caused a loss of weight in rats and rabbits. It does not cause elevation of body temperature, increase in basal metabolism or diarrhea.

2. The acid-insoluble fraction caused much greater weight losses, severe diarrhea, elevation of body temperature, increased heart rate, rapid respiration, and a rise in basal metabolism.

3. If artificial digestion has not destroyed any of the physiological activity, at least only half of the total iodine in the thyroid is combined with the active principle.

I wish to take this opportunity to thank Mr. G. F. Stewart for making the pH adjustments for me. It has been a great opportunity and a pleasure to carry out this work under the direction of Dr. A. J. Carlson, whose advice and encouragement have meant so much.

BIBLIOGRAPHY

(1) Bubnow. Zeitschr. f. physiol. Chem., 1884, viii, 1.
(2) Langendorff. Quoted from Hutchison.
(3) Gourlay. Journ. Physiol., 1894, xvi, 23.
(4) Baumann. Zeitschr. f. physiol. Chemie, 1895, xxi, 319.
(5) Hutchison. Journ. Physiol., 1896, xx, 474.
(6) Hutchison. Journ. Physiol., 1898–99, xxiii, 178.
(7) Tamback. Zeitschr. Biol., 1898, xxxvi, 549.
(8) von Cyon and Oswald. Pflüger's Arch., 1901, lxxxiii, 199.
(9) Oswald. Arch. f. exper. Path. u. Pharm., 1908, lx, 115.
(10) Nürnberg. Biochem. Zeitschr., 1909, xvi, 87.
(11) Pick and Pineles. Zeitschr. exper. Path., 1909–10, vii, 518.
(12) Cameron and Carmichael. Trans. Roy. Soc. Canada, 1926, xx, 307.
(13) Harington and Salter. Biochem. Journ., 1930, xxiv, 456.
(14) Kendall. Journ. Biol. Chem., 1915, xx, 501.
(15) Harington and Randall. Biochem. Journ., 1929, xxiii, 373.
(16) Kunde. This Journal, 1927, lxxxii, 195.

MANGANESE AS A FACTOR IN REPRODUCTION[1]

J. T. SKINNER, EVELYN VAN DONK[2] AND H. STEENBOCK

From the Department of Agricultural Chemistry, University of Wisconsin, Madison

Received for publication May 25, 1932

Because of its low content of certain inorganic elements, notably iron, copper and manganese, whole milk has been used extensively as a basal ration in studies of the functions of these elements in animal nutrition. Painstaking attempts to produce basal rations lower in these elements by using purified food materials end frequently in synthetic rations in which these elements are present in greater concentrations than in cow's milk.

For various reasons, some of which have been satisfactorily explained, milk does not support normal reproduction. In 1925 Daniels and Hutton (1) reported that reproduction in milk fed animals was benefited by the addition of small amounts of certain inorganic elements. It is now known that iron and copper must be added to a milk diet in order to maintain the hemoglobin level which, obviously, is necessary if normal reproduction is to be secured. Waddell, Steenbock and Hart (2) found that reproduction was far below normal on a milk diet supplemented with iron and copper, and Krauss (3) secured no young from rats reared on such a diet. Keil and Nelson (4), obtained young from females receiving milk supplemented with copper and iron, but observed that lactation was frequently inadequate to permit the mothers to rear their offspring.

One phase of reproduction which lends itself readily to accurate observations over even relatively short periods of time is that of oestrum. Observations by Waddell et al. (2) on the oestrous cycle in the rat when receiving iron and copper as supplements to whole milk, indicated that oestrous cycles, if they occur at all, do so at rather long and irregular intervals. Kemmerer, Elvehjem and Hart (5) noted similar performances by female mice on such a ration. In both of these investigations it was observed that manganese, when added to the milk-iron-copper diet increased the frequency of oestrum. The conclusions regarding the effect of manganese upon the oestrous rhythm in the rat (2), unfortunately, were based upon preliminary observations in which an adequate number of animals was not available, and no controls were run simultaneously. The work de-

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] E. R. Squibb and Sons Fellow.

scribed herein is a continuation of this phase of the study of reproduction, in which sufficient animals have been used to warrant more positive conclusions.

EXPERIMENTAL. For these experiments 37 young female rats were selected from litters reared by mothers which were put on an exclusive milk diet when the young were 12 days of age. This precaution was observed in order that the animals would have the least possible stores of manganese at the beginning of the experimental period. Twenty-eight of them were placed in the control group, which received milk *ad libitum* plus 1.0 mgm. of iron and 0.5 mgm. of copper per 100 cc. The other 9 received in addition to the diet of the controls, manganese to the extent of 1.0 mgm. per 100 cc. of milk. The iron was fed as ferric chloride and was prepared from standardization iron wire according to the usual procedure (6). The C. P. grades of the sulfates of copper and manganese were employed.

One of the criteria used in determining the effect of Mn was that of incidence of sexual maturity. Mention has been made elsewhere (7) of the relative rates of growth of the two groups of animals during the first 10 weeks on the experimental rations. Since sexual maturity is usually retarded in animals which do not grow normally, it was expected that in animals receiving the manganese supplement the vaginal orifice would be established at an earlier age than in the controls. Of the 28 females in the control group 5 were changed to a second supplement before this stage of development had been reached; therefore the comparison using this as a criterion must be limited to the performance of the remaining 23.

Examination of the growth curves in charts I and II shows that there were outstanding exceptions to the relationship which usually exists between the rate of growth and attainment of sexual maturity. For the 23 females maintained on the milk-iron-copper diet the average age attained before the vagina opened was 100 days; the limits were 60 and 188 days. The average would have been greater had the other five been continued on the control diet, for they were 128 days old when changed to the supplemented diet. The age of the manganese animals ranged from 61 to 131 days and averaged 87 days when the vaginal orifice was established. Opening of the vaginal orifice prior to the hundredth day was noted in only 39.2 per cent of the controls as compared with 66.6 per cent of those receiving manganese.

In chart I are superimposed on the growth curves a record of the oestrous cycles of 12 animals which were maintained on the control diet, and 9 which received an additional supplement of manganese. In order to conserve space and since our primary interest is in presenting the frequency of oestrum, the growth curves during the first 60 days of life have not been included.

A study of chart I reveals that, on the whole, oestrum was poor when iron

and copper comprised the only supplement for milk. Animal 132 was an outstanding exception from the beginning, she having matured early and exhibited oestrum at regular intervals over a period of 4 months. In addition to this female, 6 others also showed fairly regular oestrum during the

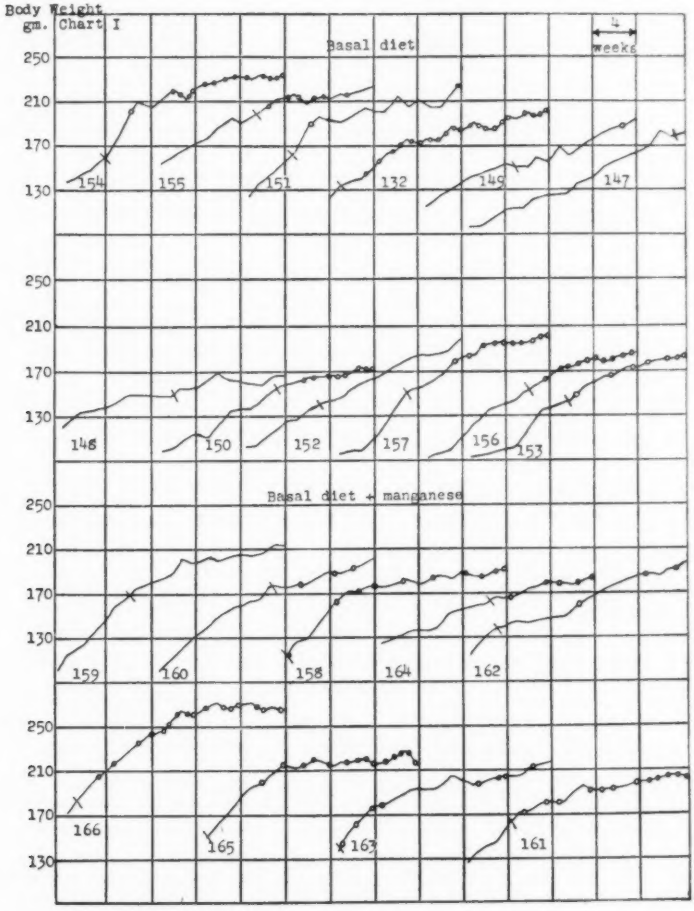


Chart I. Effect of manganese on oestrum. Basal diet: milk *ad libitum;* iron, 1.0; and copper 0.5 mgm. per 100 cc. of milk.

Supplement: manganese 1.0 mgm. per 100 cc. of milk.

In this and the following chart the cross lines on the curves indicate establishment of the vaginal orifices.

It is evident that manganese was ineffective in producing normal oestrum.

last 2 months of the experiment, but the average cycle for this period was decidedly longer than normal, viz., 7.5 days. It is improbable that these

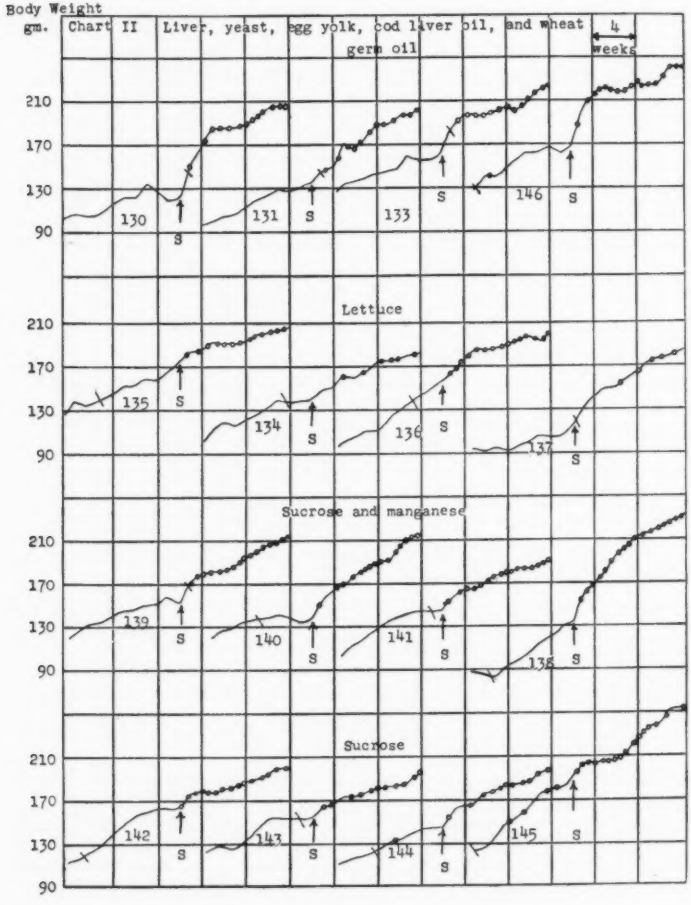


Chart II. Basal diet: milk *ad libitum;* iron, 1.0; and copper 0.5 mgm. per 100 cc. of milk.

Supplements at points *S:* 1. food mixture consisting of liver, 3; yeast, 2; egg yolk, 2; cod liver oil, 0.5; and wheat germ oil, 0.5 gram per 100 cc. of milk. 2, Five grams of lettuce per animal daily. 3, Ten grams of sucrose and 1.0 mgm. of manganese per 100 cc. of milk. 4, Ten grams of sucrose per 100 cc. of milk.

The sucrose-manganese supplement was most effective in promoting normal oestrum. Each of the other supplements, although to a lesser extent, also tended to promote a normal oestrous rhythm.

females obtained additional substance or substances by way of contamination since they were caged with other animals in which oestrum never occurred. The difference in behavior must therefore be attributed to variations in individuals. Further evidence that animals on a milk-iron-copper diet do not show the normal oestrous cycle was obtained on the other 16 animals which, prior to the last 10 weeks, were kept on this diet. Mention will be made of them later in connection with a discussion of chart II.

As is evident in the lower half of chart I, where the curves for the manganese supplemented group are given, the effect of this element was not what would have been expected from the work cited above (2). Indeed there was little difference between the oestrous rhythm in this group and the control group. Although a greater percentage of these animals exhibited oestrum three or more times, there were some control animals which performed as well as any which received manganese.

Effect of various food materials on oestrum. When it became apparent that additions of manganese to the milk-iron-copper diet were ineffective in regularly promoting normal oestrum, it was decided to try substances other than single mineral elements. Consequently, 16 of the animals which had been reared in the control group and which had shown no indication of normal oestrum were divided into 4 groups and the control diet was supplemented as follows: 1, a food mixture consisting of liver, yeast, egg yolk, cod liver oil, and wheat germ oil; 2, head lettuce; 3, sucrose and manganese; and 4, sucrose alone. In the selection of these supplements we were guided by previous work in this laboratory (8) which had indicated that oestrum was more regular in animals on a milk-iron-copper diet when the caloric intake was increased. An effort was made to supply the first group with additional energy, potent sources of the known vitamins, and an animal food rich in minerals. Enough of the supplemental mixture was prepared at a time to last 3 or 4 weeks and stored at a temperature below freezing, the daily requirement being removed as needed. The supplement of the second group, viz., head lettuce, should have furnished any requisite supplementary minerals as well as small amounts of some of the vitamins. Unlike the first two supplements which might have contained one or more specific substances requisite for normal oestrum, the other two supplements were designed primarily to increase the energy intake. The possibility that a pronounced response to such treatment might depend upon the presence of more manganese in the diet than the milk would provide led us to include this also in one of the supplements. The performances of these 4 groups are shown in chart II.

It is evident from a study of the upper half of the chart that frequency of oestrum was increased when either the food mixture or lettuce was added to the basal diet. During the 10 week period that the 4 animals were on the former oestrum occurred every 5 days, due allowance being

made for the time elapsing previous to establishment of the vaginal orifice. Previously the one sexually mature animal had exhibited oestrum only once over a period of 2 months and the remaining three, although 128 days of age when the supplement was added, were yet sexually immature. It will be noted that each of these reached maturity within a week after the addition of the supplement. Lettuce proved less effective than the food mixture. Whereas in two of the animals oestrum began to occur regularly soon after the addition of lettuce, the performance of the other two was subnormal throughout the entire period. However, it should be noted that oestrum had not occurred in either of the 3 sexually mature animals while they were on the exclusive milk-iron-copper diet.

The performances of the animals in both groups receiving sucrose, which are recorded in the lower half of chart II, indicate that for normal oestrum the prime deficiency of the milk-iron-copper-ration is that of the energy. Whereas in the sucrose-manganese group oestrum had not been observed in any animal prior to the addition of this supplement, the average rate of appearance of oestrum thereafter was once every 4.3 days. Animals receiving sucrose but no manganese exhibited somewhat longer, though regular, cycles of 5.4 days' duration. It should be mentioned that sucrose was found to contain only 0.021 mgm. of manganese per kilo. Actually the total manganese intake of the animals which received the sucrose supplement was less than that of the controls due to a compensatory decrease in the consumption of milk by the former. It is therefore evident that energy was the prime determinant. However, the maximum supplementary effect of sucrose was not elicited without manganese additions.

Records which were obtained during the last 26 days of feeding show that the approximate intake of energy per animal in each group during this period was: control, 830; manganese, 840; food mixture, 880; lettuce, 930; sucrose-manganese, 1130; and sucrose, 1110 Calories.

It is unfortunate that no records were kept of the food consumption of the various groups for the periods immediately following the change of supplements. Greater differences in the amounts of food ingested may have occurred at that critical stage than later in the experiment. The data, as far as they go, show that the animals on the sucrose and sucrose-manganese supplements, in which oestrum was regular, consumed approximately $\frac{1}{3}$ more calories than the controls. On the other hand, this relationship does not hold for the other groups in which the frequency of oestrum was increased, and our present information permits no conclusion for these groups as to the factor or factors responsible for the improvement.

SUMMARY

Female rats reared on whole milk fortified with copper and iron did not attain sexual maturity, as indicated by establishment of the vaginal orifice, as early as those receiving the same ration supplemented with manganese.

Females receiving a manganese supplement did not exhibit normal oestrous cycles. Like those on the milk-iron-copper diet they often failed to exhibit oestrum over long periods of time and when cycles occurred, they were less frequent than in the normal animal.

Females receiving sucrose and manganese in addition to the basal ration exhibited normal oestrous cycles of 4.3 days' duration. Those given sucrose but no manganese performed less satisfactorily. When a food mixture (liver, yeast, egg yolk, cod liver oil and wheat germ oil) was added to the basal diet, oestrous cycles occurred at intervals slightly longer than normal, namely, 5 days.

BIBLIOGRAPHY

(1) Daniels, A. L. and M. K. Hutton. Journ. Biol. Chem., 1925, lxiii, 143.
(2) Waddell, J., H. Steenbock and E. B. Hart. Journ. Nutri., 1931, iv, 53.
(3) Krauss, W. E. Journ. Dairy Sci., 1929, xii, 242.
(4) Keil, H. L. and V. E. Nelson. Journ. Biol. Chem., 1931, xciii, 49.
(5) Kemmerer, A. R., C. A. Elvehjem and E. B. Hart. Journ. Biol. Chem., 1931, xcii, 623.
(6) Waddell, J., H. Steenbock and E. B. Hart. Journ. Biol. Chem., 1929, lxxxiii, 243.
(7) Skinner, J. T., W. H. Peterson and H. Steenbock. In press.
(8) Van Donk, E. and H. Steenbock. In press.

I. THE EFFECT OF EXPERIMENTAL HYPERTHYROIDISM ON GASTRO-INTESTINAL MOTILITY[1]

DOROTHY FETTER AND A. J. CARLSON

From the Physiological Laboratory of the University of Chicago

Received for publication May 26, 1932

Hyperthyroidism as it is observed in the clinic is frequently accompanied by gastro-intestinal symptoms, the most common of which are increased desire for food, and diarrhea. Since in hyperthyroidism oxidation is increased, more food is necessary than normal in order that the tissues of the body should not be used. A hyperthyroid patient may require a diet containing as many as 5,000 calories in order to maintain his body weight (1). Exercise of any kind is accomplished with a greater expenditure of calories than normal. Therefore it may be assumed that the increased desire for food is an adjustment to a new bodily demand, and is a generalized sensation. Carlson has shown that hunger is a sensation accompanying movements of the empty stomach (2); in conditions where hunger is abnormally keen, contractions of the stomach are increased in height and frequency (3). Presumably then the increased desire for food in hyperthyroid patients may be related to an increased motor activity of the empty stomach. Perussé and Rozen (4) have studied the activity of the empty stomach of dogs during periods of feeding with desiccated thyroid, and found that the motility varied, depending upon the amount of thyroid administered. Kratinoff (5) has also studied the effects of induced hyperthyroidism upon the activity of the stomach and duodenum of dogs, and found in some cases an elevation, and in some cases a depression of the motility.

The diarrhea of hyperthyroidism also indicates an increased motility of the digestive tract. King (6) mentions diarrhea as one of the most frequent and annoying symptoms of the disease. He attributes it to the lack of free hydrochloric acid which allows the pylorus to stay open and food to pass on before digestion has occurred, thereby giving greater stimulation to the intestinal tract. Eppinger and Hess (7) attribute the diarrhea to the increased activity of the vagus nerve. Crotti (8) says that the stomach of goiter patients is in a state of constant spasticity as observed fluoroscopically. Baker (9) mentions diarrhea as a common

[1] This work has been aided by a grant from the Rockefeller Foundation Biological Fund, University of Chicago.

symptom in exophthalmic goiter. Lockwood (10) finds diarrhea present in only 4.4 per cent of the 90 cases with an average basal metabolic rate of +35 which he studied. Urmössey and Lukacs (11) found that in 15 of 24 cases gastro-intestinal motility of babies was speeded up by treatment with thyroid material. Deusch (12) also finds that thyroglandal injections increase intestinal motility as revealed by the fluoroscope.

Our work includes studies of the effect of thyroid feeding on the motor activity of the gastro-intestinal tract of dogs. We studied the effect of thyroid feeding on the gastric contractions of the empty stomach, and also on the movement of food through the digestive canal.

METHODS. The experiments to be described were run on six dogs. The dogs were kept on a constant diet consisting of 250 grams of meat, 250 cc. of milk, and 200 grams of bread per day. On this diet the dogs maintained their normal weight. The general condition of the dogs was watched, and the weight and temperature were recorded every other day. On one dog basal metabolism tests were frequently taken.

The activity of the gastro-intestinal tract was studied when the stomach was empty by means of the balloon method. The dogs were trained to swallow a stomach tube with a condom balloon attached and the records of the contractions of the empty stomach were taken according to the method suggested by Boldyreff and perfected by Carlson. A bromoform monometer was used, and the pressure at the beginning of each experiment was one centimeter of bromoform. The motility was recorded for a period of three hours, beginning 20 or 24 hours after the dogs had been fed.

The progress of food through the digestive tract was observed by means of the fluoroscope. Once a week, instead of the usual meal, the dogs were fed 200 grams of ground meat mixed with 70 grams of barium sulphate. Note was taken 1, of the time when food first left the stomach; 2, of the time when material entered the ascending colon; 3, of the emptying time of the stomach; 4, of the emptying time of the colon.

The gastric motility was studied by the above methods on the normal dogs for a period varying from two weeks to one month. The dogs were then fed daily 0.4 gram per kilo body weight of Armour's desiccated thyroid in addition to the usual meal. On this amount of thyroid the body temperature rose and the weight decreased. The dog on whom basal metabolism studies were made showed a basal rate increased to +50 per cent. The dogs showed increased nervousness, if restlessness, irritability of temper, and twitchings of the muscles are indications of this condition. Although the water balance was not measured, it was obvious that the dogs drank far more water than normally and urinated more copiously. All the dogs showed an increased desire for food; three showed diarrhea (13). By means of these symptoms it can be seen that the condition in dogs during the feeding of thyroid material resembles spontaneous hyperthy-

roidism in man. The hunger contractions and the progress of the barium meal were studied throughout the period of thyroid feeding. This period varied from one to two months depending on the time necessary for the symptoms to manifest themselves, and their severity.

After thyroid feeding was discontinued, the gastro-intestinal activity was studied for a period of from one to two months.

RESULTS. 1. *Gastric contractions.* In our six dogs, the normal type of gastric contractions varied. Three of these dogs showed low motility, described by Carlson as the 20 second rhythm (14), type 1, or complete rest of the stomach. On the remaining three dogs vigorous type 2 contractions (15) were usually observed.

The gastric motility of all dogs definitely increased over the normal during the period of induced hyperthyroidism. Records of higher activity were obtained about a week after the ingestion of thyroid was initiated. The length of the hunger periods and the height of the individual contractions were increased, also periods of increased tonus became frequent. A vigorous type of activity showing type 2 contractions on which were superimposed smaller contractions of the 20 second rhythm was observed frequently in all dogs during the "hyperthyroid" period.

After the thyroid feeding was discontinued, gastric motility remained high for a few days (average, six) and thereafter dropped. In two dogs the motility steadily decreased to the normal level and remained there. In the other dogs, while the motility decreased below that observed in the hyperthyroid phase, it did not drop to the normal level except occasionally. Usually the contractions were higher than those observed before thyroid was fed, and occasionally high contractions like those observed during the "hyperthyroid" period occurred as long as two months after the thyroid feeding had been stopped.

It is interesting to note that the gastric motility of the dog on which basal metabolism tests were run never reached the normal level during the two months that contractions were recorded following the feeding of thyroid. However, the basal metabolic rate fell from +50 per cent to the normal at the end of the first week after the thyroid feeding was discontinued, and remained there.

2. *The gastro-intestinal activity as observed by the fluoroscopic method.* The progress of the barium meal through the gastro-intestinal tract of the dogs showed a greater uniformity than the contractions of the empty stomach. In four of the dogs the barium meal usually stayed in the stomach until late during the first hour after its ingestion, or during the second hour, when small particles could be observed in the intestines. Food usually reached the ascending colon five hours after feeding. The complete emptying time of the stomach varied between 5 and 10 hours. The colon was not emptied of barium until the next day, about 24 hours

after its ingestion. Two of the dogs showed intestinal rates varying considerably from the above description.

One of these dogs showed a faster normal rate. Barium was always observed in the small intestine within the first hour after feeding. It always reached the ascending colon between the third and fourth hours after feeding. The emptying time of the stomach was variable, occurring between 6 and 10 hours after feeding. The time of defecation was also variable, barium frequently being given off 2 or 3 hours after it had reached the descending colon. The bulk of the material was not expelled until the following day, however.

One dog showed extremely slow progress of the food through the digestive tract. In only one instance out of four observations was barium observed in the small intestine during the first hour after ingestion. It did not reach the ascending colon until 5 or 6 hours later. The stomach normally showed the presence of barium in small amounts 10 to 11 hours after feeding. In one instance only the stomach was completely empty 8 hours after feeding.

It is interesting to note that the dog whose intestinal rate was abnormally fast was one of those animals showing high gastric motility as revealed by the gastric hunger contractions; and that the dog whose intestinal rate was slower than the others showed low gastric hunger contractions during the period previous to thyroid feeding.

During the period of thyroid feeding increased gastro-intestinal motility was shown by means of the fluoroscope in five of the six dogs. Appreciable quantities of barium always appeared in the intestine during the first hour after its ingestion. Barium appeared in the ascending colon 1 to 2 hours earlier than in the normal condition, or 3 to 4 hours after ingestion. While the emptying time of the stomach appeared to be variable according to the method of observation, the stomach was always emptied at least by the shortest time limit observed in the normal condition. In all except one dog, barium remained in the colon for 24 hours after feeding, as before. In the exceptional dog, the bulk of the barium was always in the descending colon in 8 hours and defecation occurred at the tenth hour in five out of six observations. The stools of this dog were always very soft or fluid in consistency during the period of "hyperthyroidism." In two other dogs the stools were softer in consistency than normal during the "hyperthyroid" period, but nevertheless the barium was retained the normal length of time in the colon.

The dog whose intestinal rate has been described as unusually rapid normally showed no appreciable change during the period of induced hyperthyroidism. However, in this dog, the activity of the empty stomach was increased as recorded by the balloon method.

The intestinal rates of the five dogs which increased during the "hyper-

thyroid" period returned to the previous normal level within two weeks after the thyroid feeding was discontinued.

Discussion. Perussé and Rozen (4) report an increase in the height of gastric contractions in dogs fed daily 2.5 grams desiccated thyroid per kilo body weight. When the dose is increased to 5 grams per kilo body weight they note a preliminary period of depression followed by an increase in height of the gastric contractions. With a 15 gram dosage they report no constant increase in hunger contractions.

Kratinoff (5) reports in four of the six dogs in which he studied the effect of "hyperthyroidism" on gastric contractions, an increase in the hunger contractions during the period of thyroid administration, whether the thyroid was given in one single large dose, or fed daily in small amounts, or whether thyroxine was injected. In two of the six dogs, he reports a depression of the hunger contractions under similar conditions.

All of our six dogs showed an increase in hunger contractions during thyroid feeding, but the effect of administering thyroid in varying doses was not studied. The dose used (0.4 gram per kilo body weight) brought on symptoms similar to those seen in the spontaneous hyperthyroidism of man, including an increase in the basal metabolic rate. Increased gastric motor activity was observed to accompany this condition in our animals.

Kratinoff reports that ten months after daily feeding with 1 gram of desiccated thyroid for a period of thirty days, the gastric contractions were often as high as during the "hyperthyroid" period. He finds that the stomach behaved erratically after "hyperthyroidism," at one time showing a depression in motility, and at another time very high contractions. He concludes that the thyroid has a long continued effect on gastric motility, which he did not explain.

We found a decrease in the activity after the thyroid was discontinued, but we occasionally observed the type of contractions, characteristic of the "hyperthyroid" condition. After two months, the gastric activity had returned to the normal level in only two dogs. The explanation of this long continued action of the thyroid on the stomach is difficult. All other hyperthyroid symptoms disappeared. The dogs gained weight, frequently above the normal level. The nervousness, the elevated body temperature decreased, the polydipsia, and polyurea disappeared; and in the dog on whom basal metabolic studies were made, the basal returned to the normal. We cannot assume therefore that any abnormal amount of functioning thyroid material remains incorporated in the protoplasm long after the thyroid intake is stopped. Careful studies on the blood chemistry of dogs, the effect of low protein and cholesterol values on the gastric contractions, and a study of these values in the blood of dogs long after periods of thyroid feeding might throw some light on these conditions.

Not only is the digestive tract shown to be more active during the time of thyroid feeding by the gastric contractions but also by the barium meal. Inasmuch as diarrhea is a frequent symptom of hyperthyroidism in man, one might expect to find that the barium residue would go through the colon at a greater rate of speed than the normal, so that water absorption would be prevented. This expected condition was actually observed in only one of the six dogs studied. In this dog the stools were definitely of more fluid consistency than the normal. In two other dogs softer stools than normal were observed, but in these dogs the barium residue was retained the usual length of time. In the three other dogs the barium remained in the colon the normal length of time and no symptoms of diarrhea were manifested.

In all dogs except one, however, an increased speed of the barium meal through the stomach and small intestine was noted.

Why the increase in speed of the barium meal should occur in the upper part of the digestive tract is difficult to explain. If the thyroid should act by means of the vagus nerve this effect would be expected, but later studies indicate that the thyroid does not act in this manner. Furthermore, the fact that gastric acidity is decreased rather than increased during hyperthyroidism (17), (18), (19) does not indicate over activity of the vagus. McCann (20) has lately investigated the factors which might influence the emptying time of the stomach. He finds that free hydrochloric acid or the products of digestion have no effect on the emptying time. He finds in the first phase of digestion a limited emptying of the stomach accompanied by great tonic and peristaltic activity of the antrum. As the antrum relaxes in the later phases of digestion, the emptying time decreases. McCann thinks that the activity of the antrum depends on the irritability and stimulating action of raw protein to vigorous tonic and peristaltic contractions. He also thinks that the change of the meal to the more fluid state is a factor influencing the emptying time of the stomach. Should one assume then that the thyroid acts by directly stimulating the muscle to greater activity, one might conclude that one should find delayed emptying of the stomach since the higher activity of the antrum tends to keep food within it, according to McCann. However, the contrary is true. Greater activity of the muscles of the intestinal tract would bring about an increased rate of speed with which barium entered the large intestine, and would therefore explain that finding.

Explanation of the increased intestinal activity might lie in the change in gastric secretion. Since gastric acidity is lessened in hyperthyroidism protein food is probably not as well digested as in the normal dog, and larger pieces of undigested food might pass into the small intestine acting as a stimulus to peristaltic activity.

SUMMARY

1. Daily feeding of 0.4 gram per kilo body weight of desiccated thyroid per dog increases the activity of the empty stomach of dogs as shown by the type and height of the contractions of the empty stomach. After the thyroid feeding is discontinued there is a lowering of gastric activity, although in two months a return to the normal level was not observed in two dogs.

2. During the period of thyroid feeding the emptying time of the stomach is decreased, and a barium meal passes more rapidly through the small intestine. After the administration of thyroid is stopped, the speed of the barium meal through the digestive tract returns to its former rate.

BIBLIOGRAPHY

(1) Boothy, W. M. and I. Sandiford. Journ. Amer. Med. Assoc., 1923, lxxxi, 795.
(2) Carlson, A. J. The control of hunger. University of Chicago Press. 1916, chaps. 3 and 4.
(3) Carlson, A. J. Ibid., chap. 16.
(4) Perussé and Rozen. This Journal, 1929, xci, 291.
(5) Kratinoff. Zeitschr. f. d. gesammt. exp. Med., 1929, lxiv, 376.
(6) King, C. H. Med. Clin. N. Am., 1919, ii, 1655.
(7) Eppinger and Hess. Zeitschr. klin. Med., 1909, lxvii, 231.
(8) Crotti, A. Thyroid and thymus. Lea & Febriger, Philadelphia, 1918.
(9) Baker, L. F. Trans. Amer. Gastro-Enterol. Assoc., 1918, May 7.
(10) Lockwood. Journ. Amer. Med. Assoc., 1925, lxxxv, 1032.
(11) Urmössy, E. and J. Lukacs. Arch. f. Kinderh., 1930, lxxxix, 161.
(12) Deusch, G. Deutsch. Arch. klin. Med., 1923, cxlii, 1.
(13) Kunde, M. M. This Journal, 1927, lxxxii, 195.
(14) Carlson, A. J. Op. cit., p. 36.
(15) Carlson, A. J. Ibid., p. 44.
(16) Hardt. This Journal, 1916, xl, 314.
(17) Truesdell. This Journal, 1927, lxxvi, 20.
(18) Chang and Sloan. This Journal, 1927, lxxx, 732.
(19) McCann. This Journal, 1929, lxxxix, 497.

II. THE EFFECT OF INDUCED HYPERTHYROIDISM ON THE GASTRO-INTESTINAL MOTILITY OF VAGOTOMIZED DOGS[1]

DOROTHY FETTER, LOUIS BARRON AND A. J. CARLSON

From the Physiological Laboratory, University of Chicago

Received for publication May 26, 1932

Previous studies have shown that dogs which are fed daily 0.4 gram per kilo body weight of desiccated thyroid show an increase in the contractions of the empty stomach, and an increase in the speed with which a barium meal passes from the stomach to the colon. Clinical observations and experiments show that the motor activity of the digestive tract is increased during hyperthyroidism. Various explanations have been given for this.

Möbius (1) thinks that the thyroid secretion works directly on the intestinal wall. Wolpe (2) assumes that the lack of free hydrochloric acid observed in many hyperthyroid cases is responsible for the diarrhea of hyperthyroidism. Deusch (3) thinks that the thyroid hormone acts on the nervous mechanism of the intestine, while Eppinger and Hess (4) attribute the diarrhea of hyperthyroidism to overactivity of the vagus nerve.

The following studies were undertaken in order to see whether the vagus nerve is responsible for the increase in the motor activity of the stomach and small intestine which we observed in our previous studies. The anterior and posterior branches of the vagus nerve were sectioned just below the diaphragm in four dogs. The gastric hunger contractions, and the motor activity of the digestive tract as shown fluoroscopically were studied for two months following the vagotomy. Four-tenths gram of desiccated thyroid per kilo body weight was given the dogs daily, and the gastro-intestinal activity studied as before. After one to two months the feeding was discontinued and the activity of the digestive tract was studied for two months longer. The methods used for these studies were described in paper I.

To make sure that the vagus nerve was cut and did not regenerate during the time of the experiment, the effect of insulin on the gastric contractions was observed. Insulin increases the gastric contractions of the normal animal (5) and decreases those of the vagotomized dog (6). At the conclusion of the experiment, the effect on the digestive tract of stimulating

[1] This work has been aided by a grant from the Rockefeller Foundation Biological Fund, University of Chicago.

the vagi above the point of section was noted. Contraction of the stomach or intestine on stimulation of the vagi shows that some fibers are functioning. No activity of the gastro-intestinal muscles on stimulation of the vagi indicates that the nerves are sectioned and have not regenerated.

RESULTS. *Dog. 1.* 1. *Gastric contractions.* The contractions of this dog when vagotomized are usually type 1 (7). The interval between contractions varies between two and ten minutes, thereby differing from normal type 1 contractions where the interval is usually about one minute. The type of contraction observed in dog 1 is characteristic of vagotomized dogs (8). Frequently 20 second rhythms (9) are noted with a height of ½ to 1 cm.

For about ten days after the feeding of thyroid had been started, the gastric contractions showed no change from the normal. Then a slight evidence of increasing hunger contractions was observed. Type 1 contractions were seldom noted and the 20 second rhythms predominated. The 20 second rhythm reached 2 or 3 cm. in height with occasional periods of type 2 contractions interrupting.

The "hyperthyroid" type of contractions continued for about a month after thyroid feeding had been stopped; then the normal type 1 contractions became the predominating type observed.

Dog 1 showed by the balloon method a slight increase in gastric activity during the period of thyroid feeding.

2. *The gastro-intestinal activity as observed by the fluoroscopic method.* In four of the seven observations made two months subsequent to the vagotomy, food was not seen in the intestine until three or four hours after its ingestion, a considerably longer time than was ever observed in the unoperated dogs. A small shadow was usually noted in the stomach on the day following the taking of barium. This was never observed in the normal dog. Barium reached the ascending colon six or seven hours after feeding. Defecation of barium occurred at a period varying between twenty-four to thirty-one hours after its ingestion.

After thyroid feeding was initiated a great increase was noted in the speed of the barium meal throughout the digestive tract. Food was always observed in the small intestine at the second hour after feeding. During the first two weeks of thyroid feeding, faint shadows were observed in the stomach twenty-four hours after the ingestion of barium; thereafter the stomach emptied itself within the first ten hours. During the first two weeks of thyroid administration, barium was observed in the ascending colon no earlier than six hours after ingestion. After two weeks of thyroid feeding the dog developed diarrhea. Defecation of barium occurred shortly after it reached the descending colon, and continued to be passed at intervals as more accumulated in the rectum. The last remnant of barium was passed during the night or on the morning after its ingestion.

After thyroid administration ceased, the stomach continued to show the same type of activity as during the "hyperthyroid" phase; the intestinal activity lessened, however. Barium was often observed in the small intestine within the first hour after its ingestion. Readings made twelve hours after the taking of barium always revealed shadows in the stomach, but for seven weeks the stomach was completely empty twenty-four hours after feeding. Food was not observed in the ascending colon until five or six hours after its intake, however, and the diarrhea disappeared. Defecation of normally formed barium stools occurred the morning after the material had been taken.

Dog 1 was operated June 4. The effect of insulin on the hunger contractions was noted August 9 and October 24. In both cases the hunger contractions ceased within an hour after the injection of insulin, and no increase in the contractions was noted while the record was taken. This indicates that the vagus nerves to the stomach were not functioning. On November 8, five months after operation, the vagi were stimulated electrically in the neck, and the effects on the stomach observed. No gastric or intestinal activity occurred on stimulation, therefore we assumed that the fibers of the vagus leading to the digestive tract had been successfully sectioned, and no regeneration had taken place during the course of the experiment.

Dog. 2. After vagotomy dog 2 showed a decided decrease in the gastric contractions as compared to those observed in the normal. The normal type of motility previous to vagotomy had been type 2 (11) rising to a height of $2\frac{1}{2}$ to $5\frac{1}{2}$ cm. After vagotomy, 20 second rhythm rising to a height of only $\frac{1}{2}$ to 1 cm. was frequently observed. Often for three hours only low tonus changes were recorded. Type 1 motility also occurred with the interval between contractions varying between three and ten minutes.

After thyroid feeding was initiated, the 20 second rhythm was still predominant, although it differed from that previously observed by rising in height to 2 or 3 cm. During the second month of thyroid feeding, tonus changes were frequently associated with the 20 second rhythm, and often it was varied by falling into the 20 second rhythm interrupted by ten minute stretches of type 2 contractions. A half-hour or an hour of type 2 contractions frequently interrupted an otherwise low record of motility.

After thyroid feeding was stopped, the motility continued of the same type as we observed during the "hyperthryoid" phase for three weeks. Then activity dropped to the normal level; 20 second rhythm rising to a height of $\frac{1}{2}$ to 1 cm. predominating, with occasional records showing type 1 motility.

After vagotomy, food was observed in the stomach twelve hours after

feeding, and frequently small barium shadows were present twenty-four hours after ingestion. The stomach then retained its contents for a much longer time after vagotomy than before. However, for the first month after the section of the vagi, the intestine showed much greater activity than the normal. Whatever material escaped from the stomach passed through the small intestine quickly, and barium was observed in the ascending colon three hours after feeding. Soon after it reached the descending colon it was expelled, and defecation of barium occurred at intervals of one or two hours until it was completely gone. The stools were fluid. The dog manifested a severe diarrhea constantly during this period. A month after the operation the diarrhea stopped, and food passed more slowly through the entire intestine. It did not reach the ascending colon until four to five hours after ingestion. Defecation occurred eight hours after food intake, and the stools were firmer than previously.

After the intestinal activity had remained at a low rate for three weeks, thyroid feeding was started. The intestinal rate increased. Food reached the ascending colon usually at the third hour after its ingestion. Defecation of barium frequently occurred five hours after feeding, and the diarrhea became severe. No noticeable difference was observed in the activity of the stomach. Some barium was always seen in the stomach twelve hours after feeding, but a residue was observed the next day on only two occasions.

After the thyroid feeding was discontinued the only noticeable difference in the passage of the barium meal was that it remained an increased length of time in the colon. Defecation did not occur as a rule within the first twelve hours after eating. No difference was noted in the motility of the stomach, and the barium reached the ascending colon three or four hours after its ingestion. Four weeks after the thyroid administration was stopped, barium reached the colon four to five hours after feeding, indicating a gradual decrease in the activity of the gastro-intestinal tract.

During the course of the experiment the effect of insulin on the gastric contractions was observed. In no case was there an increase in contractions, and a decrease was observed ½ to 1 hour after the injection of insulin. On May 10, five months after vagotomy, the vagi were stimulated in the neck and the gastric activity recorded. Under light ether anesthesia all activity of the stomach had ceased, and no contractions were noted when the vagi were stimulated with the induction coil. We therefore assumed that the vagus fibers to the stomach were not functioning.

In this vagotomized dog then, the hunger contractions increased during the "hyperthyroid" period. Three weeks after the thyroid feeding was stopped the hunger contractions returned to the type of activity observed during the normal.

The results on the gastro-intestinal activity as revealed by the fluoro-

scope show an increase during the "hyperthyroid" phase over the activity just preceding it. However, the fact that motility similar to that observed while thyroid was being fed occurred subsequent to vagotomy opens the question whether or not thyroid has anything to do with the change in speed of the digestive tract.

Dog. 3. After vagotomy dog 3 showed severe intestinal disturbances. Food remained in the stomach for long periods of time. Often considerable amounts of a barium meal were observed in the stomach forty-eight hours after eating. Consistently with this slow gastric motility the dog lost weight. The weight previous to the vagotomy was 11.7 kilos, a month later it had decreased to 10.5 kilos. If this dog was fed daily it was impossible ever to find a time when the stomach was empty unless vomiting had just occurred. While some records of gastric contractions were made after vomiting, it was considered best not to interfere with such nutrition as the dog could obtain so the study of hunger contractions was not made.

Such records of gastric activity as we obtained, however, showed that either type 1 contractions or very low tonus changes occurred. In order to obtain evidence that the vagi were sectioned the effect of insulin on gastric contractions was studied. Twenty-five minutes after an injection of insulin during a period of type 1 contractions, all activity of the stomach ceased, and was not resumed while the record continued for another hour. The results indicated that the vagi were not functioning.

Studies of the activity of the digestive tract by the fluoroscopic method show low motility following vagotomy. Previous to the operation the emptying time of the stomach had been six to seven hours. After the section of the vagi, the stomach contained considerable amounts of food for two or even three days after ingestion. Barium reached the ascending colon at a time varying between five to eight hours after its intake. Defecation of some barium occurred the day after it had been fed. A month after the operation the fluoroscopic readings showed considerable decrease in the emptying time of the stomach. Some barium was usually found in the stomach twenty-four hours after feeding, but the residue was small and the main bulk of the meal had obviously passed on. Simultaneously with this improvement in gastric activity the dog gained weight and her general condition improved.

When the weight reached 11.6 kilos, thyroid was given (March 24). By April 5 symptoms of severe hyperthyroidism were manifest. The dog's weight had by this time decreased to 11.4, the temperature raised from 98° to 101° F. Polydipsia was very noticeable; the dog's water jar had to be refilled about three times daily. Accompanying the polydipsia was a noticeable polyurea. A severe diarrhea had started. The fluoroscopic observations made on April 5 and April 12 showed that barium entered the ascending colon at the third hour after food intake. Defe-

cation occurred frequently throughout the day, and the gastric intestinal tract was emptied completely about eleven hours after the barium meal had been eaten. In ten days the dog died, apparently from the effect of thyroid feeding. Autopsy revealed no abnormality of the internal organs except a hemorrhagic intestine.

Although this experiment could not be completed, we have included it here because it shows the extreme disturbance in gastric motility which may follow vagotomy. Also the great increase in motility following thyroid feeding is significant.

Dog. 4. 1. *Gastric contractions.* Shortly after the vagotomy, this dog showed a severe diarrhea which persisted for a month. At the end of that time, the dog recovered, and one month after the dog had been in good health, and his weight constant for two weeks, studies on gastric contractions showed low motility. Either type 1 contractions, occurring every three to five minutes, about 2 to 4 cm. in height were observed, or low 20 second rhythm about ½ to 1 cm. high.

Two weeks after the feeding of thyroid was initiated the gastric contractions increased in height. Increases in tonus were frequently observed. Type 1 contractions occurred often, coming every minute and reaching a height of 12 to 14 cm. After six weeks thyroid feeding was discontinued, and gastric activity as recorded by the balloon method sank to the previous level.

2. *The gastro-intestinal activity as observed by the fluoroscopic method.* Previous to thyroid feeding, the gastro-intestinal rate as observed by the fluoroscope showed that food had escaped into the small intestine during the first hour. It reached the ascending colon between the fifth and sixth hours. Fragments of barium were observed in the stomach twenty-four hours after its ingestion. The colon was emptied between twenty-four and thirty-six hours after the barium had been given.

During the period of thyroid feeding, barium entered the ascending colon two or three hours after feeding. Fragments were not observed in the stomach the next day. There was no diarrhea; the barium was not expelled from the colon until twenty-four hours had elapsed. The increased motility was observed in the upper part of the gastro-intestinal tract, as before vagotomy. A week after thyroid feeding was discontinued the gastric motility returned to its former level.

The studies on motility were continued for a month after thyroid feeding stopped. Then a study of the gastric motility following electric stimulation of the vagi above the point of section was made. No movement of the stomach or intestine following stimulations could be detected.

Discussion. The changes in the activity of the digestive tract after vagotomy are of interest. A great decrease in the activity of the stomach is shown both by the gastric hunger contractions, and by the length of

time that shadows are observed in the stomach after barium intake. Dog 3 shows that the decrease in gastric motility may reach dangerous limits. Gradually the digestive tract seems to adapt itself to the absence of the motor nerve, however.

The diarrhea following vagotomy which is reported in dogs 2 and 4 is of interest. We have observed this phenomenon in other vagotomized dogs. In two cases food was frequently defecated three hours after intake with very little change in its condition. Particles of meat were observed in the feces. In all dogs this diarrhea ceased after a month. The diarrhea might be due to changes in gastric secretion. Inasmuch as gastric acidity and secretion are lessened by the section of the vagus nerve, we may assume that digestion is impaired in vagotomized dogs. Particles of undigested food then enter the intestine and serve as a stimulus to its greater activity. In time the glands of the stomach may adapt themselves to the absence of the secretory nerve, and digestion proceeds, resulting in a disappearance of the diarrhea.

Since these vagotomized dogs show an increase in gastro-intestinal activity upon the administration of thyroid, it seems clear that the influence of the thyroid on gastro-intestinal motility is largely if not wholly independent of the possible influence of the thyroid hormone on the gastro-intestinal vagus mechanism.

SUMMARY

Upon the administration of thyroid substance, vagotomized dogs show: 1, an increase in hunger contractions; 2, an increase in the speed with which a barium meal passes through the digestive tract, particularly the stomach and the small intestines.

BIBLIOGRAPHY

(1) Möbius, P. J. Die Basedow'sche Krankheit. Vienna, 1906.
(2) Wolpe, I. M. Deutsch. Arch. f. klin. Med., 1912, cvii, 492.
(3) Deusch. Die Hyperthyreosen Handh. d. inn. Sekretion von Hirsch., 1917, pp. 1–70.
(4) Eppinger and Hess. Zeitschr. klin Med., 1909, lxviii, 231.
(5) Bulatao and Carlson. This Journal, 1924, lxviii, 148; lxix, 107.
(6) Quigley, J. P. and R. Q. Templeton. This Journal, 1929, xci, 482.
(7) Carlson, A. J. The control of hunger, p. 44.
(8) Carlson, A. J. This Journal, 1913, xxxii, 369.
(9) Carlson, A. J. The control of hunger, p. 36.
(10) Fetter, D. and Carlson, A. J. This Journal, 1932, ci, 598.
(11) Carlson, A. J. The control of hunger, p. 44.

FACTORS WHICH INFLUENCE THE FLOW AND PROTEIN CONTENT OF SUBCUTANEOUS LYMPH IN THE DOG

II. The Effect of Certain Substances which Alter the Capillary Circulation[1]

FLORENCE W. HAYNES

From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

Received for publication May 27, 1932

Previous observations on the effect on the lymph of vasodilator and vasoconstrictor substances, such as histamine, adrenin and pituitrin, have been made on thoracic duct lymph, and are summarized in table 1. Few, if any, determinations have appeared on lymph coming directly from the subcutaneous areas of the body where the effects of digestion and respiration are relatively unimportant. Recent work in this laboratory (Haynes, 1932; White, Field and Drinker, 1932) has indicated the part played by changes in arterial and venous pressure in the flow and concentration of lymph in the dog. In the present study on subcutaneous lymph, observations have been made of the effects of certain substances of general physiological significance, namely, histamine, acetyl choline, adrenin, ephedrine and posterior pituitary extract. It was hoped, by a comparison of their actions on the small blood vessels, to determine the relative importance of various capillary changes in the production of lymph.

Method. Lymph was obtained from young dogs (18 to 31 kilos) under pentobarbital-sodium, "Nembutal" (sodium-ethyl (1-methyl-butyl) barbiturate) anesthesia. The flow from the foot was measured by collecting the lymph from one or two of the main lymphatic trunks at the ankle in calibrated cannulas which were emptied every 10 minutes. Injections of the lymphatics of the dog's leg have shown that cannulation of the two main trunks on the front of the foot represents approximately one-half of the flow of lymph from the paw. In order to obtain a flow of lymph, the feet were moved passively by attaching them to a revolving wheel. Since the dogs were usually on their backs with the feet slightly above heart level, it was thought that the gradual decrease in lymph, sometimes observed for several hours after cannulation, might be partly

[1] Submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Radcliffe College.

TABLE 1

The effect of histamine, adrenin and pituitrin on thoracic duct lymph

SUBSTANCE	DOSE	OBSERVER	DATE	ANIMAL	LYMPH*		REMARKS
					Flow	Per cent protein or concentration	
Histamine	0.3 mgm. per kilo intravenously	Dale and Laidlaw	1911	Dog	+	Slight +	Anaphylaxis has been found to give the same effect (Petersen and Hughes, 1925a)
Adrenin	1:1000 clinical adrenalin intravenously	Camus	1904	Dog	+		
	Dog, 0.3 mgm. per kilo subcutaneously	Tomaczewski and Wilenko	1908	Dog and rabbit	−		Splanchnic stimulation increases lymph production (Starling, 1894)
	0.04 per cent 9 cc. in 30 min. intravenously	Bainbridge and Trevan	1917	Dog	+	Slight +	
	0.5 to 1.0 cc. of 1:1000 or a dilution	Yanagawa	1916	Dog	+	+	
		Christoni	1921	Dog	+	−	
	2 to 5 cc. 1:10,000 intravenously	Petersen and Hughes	1925b	Dog	+−	−	
	1 to 4 mgm. in 10 to 20 cc. saline intravenously	Meyer-Bisch Gunther and Bock	1926	Dog	+	+	
Pituitrin	2 to 4 cc. intravenously	Meyer and Meyer-Bisch	1921	Dog	−	+	
	2 to 3 cc. (Parke, Davis & Co.)	Bayley, Davis, Whitman and Scott	1925	Dog	−		
	0.5 cc. (Armour) intravenously	Petersen and Hughes	1925b	Dog	−	0	

* A plus indicates an increase and a minus a decrease; a plus followed by a minus indicates an increase and then a decrease.

due to the position of the dog. To avoid confusion of results with pressor substances, after which a fall in lymph flow might be expected, in a few experiments animals were placed either in the feet down position or on the side. The skin temperature was followed by a thermocouple on the shaved surface of the foot.

Observations on subcutaneous lymph from the head and neck region have been omitted since the flow of cervical lymph, as that of thoracic duct lymph, was found to follow the mechanical effects of respiratory changes much more closely than circulatory changes.

Injections were made into the cannulated jugular vein or into the femoral artery through a small cannulated arterial branch, and the blood pressure recorded from the carotid artery. At intervals samples of arterial blood were taken for hemoglobin (Sahli hemoglobinometer) and hematocrit readings. The protein content of the lymph and serum was determined refractometrically.

Results. *Histamine.* "Ergamine" acid phosphate (Burroughs, Wellcome & Co.) was injected into the blood stream in doses of 0.39 to 0.88 mgm. per kilogram, or approximately 0.026 to 0.093 mgm. per kilogram per minute, usually in a dilution of 1:1000 in saline. These doses are considerably greater than those found by Koessler and Hanke (1924) to give a perceptible fall in blood pressure in dogs (0.0027 mgm. per minute per kgm. body weight) or those used by Weiss, Robb and Ellis (1932) on humans (0.07 mgm. or 0.001 mgm. per kgm. in a single rapid intravenous injection). Although in all experiments of the present series the blood pressure fell markedly and the rate of respiration was usually increased, in only one case in which the injection was exceptionally rapid did the animal go into shock so that artificial respiration was necessary.

The lymph flow from the foot as well as the protein content of the lymph increased after sufficient doses of histamine given intravenously or when the solution was introduced directly into a branch of the femoral artery. The increase in the lymph flow was almost immediate, appearing in certain cases within two minutes after the beginning of the injection, a significant indication of the speed with which substances pass from the blood into the lymph. Although the lymph flow could be maintained at a high level only as long as injection was continued, the fact that the protein content of the lymph tended to remain above the normal level for some time as well as the appearance of red cells in the lymph in some experiments gave evidence of injury to the capillary endothelium. The change in permeability thus produced apparently has more effect on the lymph than the mere change in diameter of the capillaries.

A few measurements made of the relative amount of plasma, the percent of hemoglobin in the blood, and the concentration of the serum agree with observations in the literature that in anesthetized cats and dogs the

blood is concentrated with a loss of plasma volume after histamine (Dale and Laidlaw, 1919), and that the per cent of serum protein remains practically unchanged or falls slightly (Derer and Steffanutti, 1930).

Acetyl choline. Acetyl choline bromide (Eastman) was used in 1:200 to 1:4000 solution in doses of 0.83 to 6.8 mgm. per kilo, or approximately 0.066 to 0.42 mgm. per kgm. per minute. Injections into the jugular vein in the dog lowered the blood pressure as well as often giving indications of generalized parasympathetic stimulation such as cardiac inhibition, defecation and salivation. The skin temperature of the feet, however, fell slightly instead of rising as might be expected if the arterioles were dilated. The lymph flow decreased, if anything, and the lymph protein remained practically unchanged.

Since it is known that the action of acetyl choline is transient, it was thought that it might be destroyed before it could produce its usual vasodilator effect on the small blood vessels of the foot. For this reason injections were made directly into a cannulated branch of the femoral artery. Control experiments showed that the introduction of saline equivalent in volume to the solution used did not affect the lymph flow. Under these conditions, after acetyl choline, the skin temperature increased indicating arteriolar dilatation. The flow and concentration of lymph showed a moderate but definite increase. In no experiment was there any definite change in the per cent of protein in the serum, nor the per cent of plasma or hemoglobin in the blood.

A typical experiment is seen in figure 1. After the injection of 8 cc. of a 1:200 solution of acetyl choline into the femoral artery, the lymph flow and lymph protein were temporarily increased and the skin temperature rose sharply. One and one-half hours after the injection of acetyl choline, 8 cc. of a 1:1000 solution of histamine were similarly injected. It was assumed that since the lymph flow had returned to normal the acetyl choline had not caused permanent injury but had merely dilated the arterioles. Histamine, which dilates the capillaries and probably also injures their endothelium, caused a much greater flow of lymph and an increase in the lymph protein. This experiment would indicate, as did those of Burn and Dale (1926), that the effect of histamine on the blood vessels is more intense and persistent than that of acetyl choline.

Adrenin. Adrenin was injected at the rate of 0.0012 to 0.0045 mgm. per kgm. per minute, doses which in most cases are within or below the range of 0.0032 to 0.0037 mgm. per kilo per minute, found by Cannon and Rapport (1921) to be the rate of reflex adrenal secretion. Such injections caused in all cases a marked fall in skin temperature due to arteriolar constriction, and when injections were made intravenously a considerable rise in blood pressure. The lymph flow showed a small temporary rise usually followed by a fall, whereas the lymph protein remained practically constant.

An increase of hemoglobin in the blood and of the protein content of the serum as well as a decrease in the relative amount of plasma, observed in a few representative experiments of this series, agree with the reduction in plasma volume obtained by Nelson and Edmunds (1924) after adrenin.

Figure 2 shows graphically the results of a typical experiment of this series. Adrenin in 1:10,000 solution was injected into a cannulated

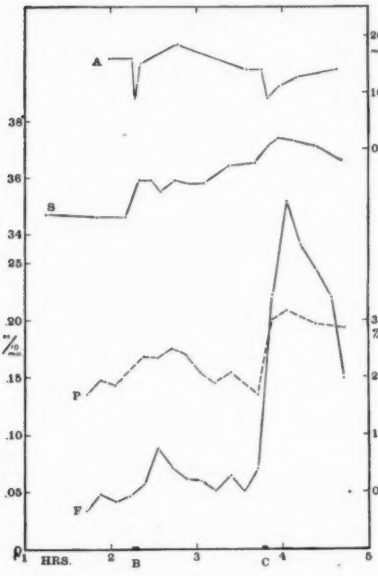


Fig. 1

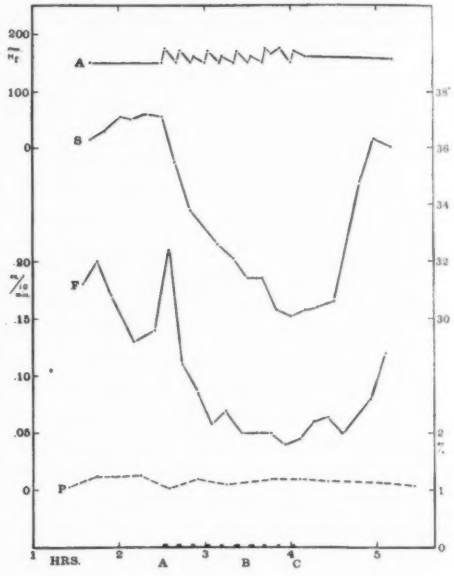


Fig. 2

Fig. 1. The effect of acetyl choline and histamine on the arterial blood pressure, skin temperature, lymph flow and protein content of leg lymph in the dog. *A*, carotid blood pressure in millimeters of mercury; *S*, skin temperature in degrees centigrade; *P*, per cent protein in lymph; *F*, flow of lymph in cubic centimeters per 10 minutes. At *B*, 8 cc. of 1:200 solution of acetyl choline were injected into the femoral artery; and at *C*, 8 cc. of a 1:1000 solution of histamine.

Fig. 2. The effect of adrenin on the arterial blood pressure, skin temperature, lymph flow and protein content of leg lymph in the dog. *A*, carotid blood pressure in millimeters of mercury; *S*, skin temperature in degrees centigrade; *F*, flow of lymph in cubic centimeters per 10 minutes; *P*, per cent protein in lymph. Lymph was obtained from the left hind leg. From *A* to *B*, at intervals indicated on the abscissa, 18 cc. of a 1:10,000 solution of adrenin was injected into the femoral artery of the left leg; and from *B* to *C*, 12 cc. were similarly injected into the right leg.

branch of the femoral artery. The lymph flow displays a slight initial rise and then a fall as the skin temperature decreases. When the temperature again rises after the effect of adrenin is over, the lymph flow again

returns to normal. The concentration of the lymph did not change, whereas the blood gave evidence of concentration.

Ephedrine. In a few experiments, ephedrine sulphate (3 per cent) was injected into the blood stream in doses of 3 to 16 mgm. per kilogram. The results are similar to those obtained with adrenin, namely, a fall in skin temperature indicating arteriolar constriction, and a temporary rise followed by a late fall in lymph flow with no marked change in the concentration of the lymph. Hematocrit and hemoglobin determinations often indicated a concentration of the blood after ephedrine.

Pitressin. The pressor fraction of the posterior pituitary extract[2] was used in a few experiments in doses of 2.0 to 7.4 pressor units per kilogram. A decrease in lymph flow usually occurred with the fall which took place in skin temperature, but the effect was less marked than that after adrenin and ephedrine.

DISCUSSION. Table 2 is a summary of all the results reported. In a separate column have been added the effects on the blood vessels recorded by other observers in order that changes in the blood and lymph may be correlated with those in the capillary circulation.

Depressor substances, as histamine and acetyl choline, increase the flow and concentration of subcutaneous lymph. In the dog the change from constrictor to dilator action after histamine takes place at the level of the small visible arteries (Burn and Dale, 1926). In contrast to acetyl choline, histamine produces dilatation of the capillaries of the dog (Abel and Geiling, 1924; Kolls and Geiling, 1924). As in man (Ellis and Weiss, 1932), more profound alterations have been found after histamine than after acetyl choline.

The pressor substances used, as adrenin, pitressin and ephedrine, have been found in general to decrease the flow of lymph without affecting its protein content. In many cases the changes in lymph flow have been found to parallel the changes in skin temperature. It might thus be concluded that a decreased flow of blood through a part, as after the injection of adrenin, is followed by less filtration into the tissue spaces and less lymph. The pressure relationships due to constriction and dilatation of arterioles or capillaries is well shown in a chart by Evans (1930). After adrenin as after histamine and acetyl choline the capillary pressure is lower than the normal. It thus appears that the change in lymph flow after these substances does not follow the capillary blood pressure but is more dependent on the state of the capillary endothelium.

[2] Pitressin containing 10 pressor units per cubic centimeter was generously supplied by Parke, Davis & Co.

TABLE 2

*The effect on subcutaneous lymph of certain substances which alter the capillary circulation**

SUBSTANCE	NUMBER OF DETERMINATIONS	LYMPH		SKIN TEMPERATURE	BLOOD PRESSURE	CONCENTRATION OF THE BLOOD	EFFECT ON SMALL BLOOD VESSELS	REMARKS
		Flow	Per cent protein					
Histamine	8	+	+	Slight + when injected into femoral artery	−	Hemoglobin + and per cent plasma −. Concentration of serum practically unchanged (Dale and Laidlaw, 1919; Derer and Steffanutti, 1930)	In dogs arterioles and capillaries dilated (Burn and Dale, 1926). Increased capillary permeability	Low body temp. Greater gland secretion. Rapidly destroyed. Possible existence normally in cells for regulation of circulation
Acetyl choline (into femoral artery—irregular or opposite effect intravenously)	4	+	Slight +	+	−+	No definite change in the present experiments	Arteries dilated in cat (Dale and Richards, 1918). Dilatation of cutaneous vessels; action prevented by atropine (Hunt, 1917)	Vagus-like inhibition of heart. Increased gastric secretion. Rapidly destroyed. Probably liberated by parasympathetic stimulation
Adrenin	7	+−	0	−	+	Per cent hemoglobin + and per cent plasma −, (Nelson and Edmunds, 1924)	Constricts mainly arterioles (Krogh, 1929)	Mobilization of red cells
Ephedrine	3	+ Late fall	0	−	Prolonged +	Per cent plasma usually −, in the present experiments. Occasionally caused hemolysis	Vasoconstriction. Action similar to adrenin (Kreitmair, 1927)	Increased salivation

Posterior pituitary extract	6	Probably late fall	0	−	+ − waves	Doubtful + in per cent hemoglobin (Kolls and Geiling 1924). No change in blood concentration (Bayley, et al., 1925). Dilution of plasma (Himwich, 1932)	In the unanesthetized dog arterioles and capillaries constrict (Kolls and Geiling, 1924)	Diuretic antidiuretic effect. Increased peristalsis

* A plus indicates an increase and a minus a decrease; a plus followed by a minus indicates an increase and then a decrease.

SUMMARY

1. The vasodilator substances, histamine and acetyl choline, increase the flow and protein content of subcutaneous lymph in the dog. The action of histamine is much more pronounced than that of acetyl choline.

2. Vasoconstrictor substances, as adrenin and ephedrine, cause an increase followed by a decrease in the flow of lymph. Pitressin has less effect but may decrease the lymph flow. The concentration of the lymph remains unchanged after these substances.

The writer wishes to express her thanks to Dr. C. K. Drinker for his interest and helpful suggestions in carrying out this work.

BIBLIOGRAPHY

ABEL, J. J. AND E. M. K. GEILING. 1924. Journ. Pharm. Exper. Therap., xxiii, 1.
BAINBRIDGE, F. A. AND J. W. TREVAN. 1917. Journ. Physiol., li, 460.
BAYLEY, E. C., J. C. DAVIS, W. WHITMAN AND F. H. SCOTT. 1925. Proc. Soc. Exper. Biol. and Med., xxii, 312.
BURN, J. H. AND H. H. DALE. 1926. Journ. Physiol., lxi, 185.
CAMUS, L. 1904. Compt. rend. Soc. de biol., lvi, 552.
CANNON, W. B. AND D. RAPPORT. 1921. This Journal, lviii, 308.
CHRISTONI, A. 1921. Arch. di fisiol., xix, 101.
DALE, H. H. AND P. P. LAIDLAW. 1911. Journ. Physiol., xliii, 182.
1919. Journ. Physiol., lii, 355.
DALE, H. H. AND A. N. RICHARDS. 1918. Journ. Physiol., lii, 110.
DERER, L. AND P. STEFFANUTTI. 1930. Biochem. Zeitschr., ccxxiii, 408.
ELLIS, L. B. AND S. WEISS. 1932. Journ. Pharm. Exper. Therap., xliv, 235.
EVANS, C. L. 1930. Recent advances in physiology. P. Blakiston's Son & Co., Inc., Philadelphia. See page 73.
HAYNES, F. W. 1932. This Journal, ci, 223.
HIMWICH, H. E. (To be published.)
HUNT, R. 1917. This Journal, xlv, 197.
KOESSLER, K. K. AND M. T. HANKE. 1924. Journ. Biol. Chem., lix, 889.
KOLLS, A. C. AND E. M. GEILING. 1924. Journ. Pharm. Exper. Therap., xxiv, 67.
KREITMAIR, H. 1927. Arch. f. exper. Path. u. Pharm., cxx, 189.
KROGH, A. 1929. The anatomy and physiology of capillaries. Yale University Press, New Haven. See page 179.
MEYER, E. AND R. MEYER-BISCH. 1921. Deutsch. Arch. f. klin. Med., cxxxvii, 225.
MEYER-BISCH, R., F. GUNTHER AND D. BOCK. 1926. Pflüger's Arch., ccxi, 341.
NELSON, E. E. AND C. W. EDMUNDS. 1924. Journ. Pharm. Exper. Therap., xxiii, 154.
PETERSEN, W. F. AND T. P. HUGHES. 1925a. Journ. Biol. Chem., lxiii, 179.
1925b. Journ. Biol. Chem., lxvi, 229.
STARLING, E. H. 1894. Journ. Physiol., xvi, 224.
TOMACZEWSKI, Z. AND G. G. WILENKO. 1908. Berl. klin. Wochenschr., xlv, 1221.
WEISS, S., G. P. ROBB AND L. B. ELLIS. 1932. Arch. Int. Med., xlix, 360.
WHITE, J. C., M. E. FIELD AND C. K. DRINKER. 1932. Unpublished.
YANAGAWA, H. 1916. Journ. Pharm. Exper. Therap., ix, 75.

GLYCOGENESIS IN THE TOTALLY PHLORHIZINIZED ORGANISM

S. G. MAJOR

From the Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minnesota

Received for publication May 28, 1932

In recent years considerable confusion has arisen in regard to the action of phlorhizin on metabolism of carbohydrate. Difference of opinion still exists as to whether the substance impairs the ability of the organism to oxidize carbohydrate, or whether excretion of sugar is dependent entirely on lowered renal threshold. In the former case, phlorhizin diabetes would be comparable with the pancreatic type, whereas in the latter it would not.

It was thought that a comparison of the glycogenetic ability of the phlorhizinized and depancreatized organisms might throw some light on the question of a comparable defect in carbohydrate metabolism, or of the two processes being due to fundamentally different mechanisms. The results of the studies on phlorhizinized animals are herewith presented. The work on depancreatized animals will be reported subsequently.

LITERATURE. Since so much work has been done on phlorhizin diabetes only those investigations dealing primarily with glycogenesis in the phlorhizinized animal will be mentioned.

Nash found the average glycogen content of the livers of phlorhizinized dogs to be higher than that of dogs of the control series. Palmer demonstrated appreciable increase in both muscle and liver glycogen in one of two dogs after preliminary total depletion of glycogen by the combination of epinephrine and phlorhizin. Paulesco and Michailesco lowered the glycogen content of their phlorhizinized animals by fasting, and two days subsequent to oral administration of glucose were able to demonstrate large quantities of liver glycogen. Sato found that phlorhizination does not abolish synthesis of glycogen in the livers of intact rabbits during infusion of glucose. Török showed that six days after administration of phlorhizin had been discontinued, and the animal had been placed on a diet of ox flesh and potatoes, the liver and muscle glycogen of the phlorhizinized dogs was unusually high. Junkersdorf found that in phlorhizinized dogs the content of glycogen of both liver and skeletal muscle was less among those animals that were killed seven hours after administration of the substance had been discontinued, than in those animals that were

killed seventeen hours later. Cori has suggested that the sugar given to a phlorhizinized animal may be temporarily stored as glycogen, even though it may be subsequently recovered quantitatively in the urine. Ringer, Dubin and Frankel expressed the belief that phlorhizinized dogs were able to synthesize glycogen from such glycogenic substances as glycine, propionic acid and lactic acid.

Csonka found no increase in the glycogen content of either liver or muscle among phlorhizinized dogs killed after administration of glucose. Nash and Benedict found that in only one of twelve phlorhizinized dogs was the content of muscle glycogen higher than that of the control series, although in another series of experiments Nash demonstrated that muscle glycogen had been formed in such animals during administration of glucose.

METHODS AND MATERIAL. The dogs used in the experiments were phlorhizinized in the following manner. On the first day of phlorhizination 1 gram of the substance, dissolved in solution of sodium carbonate, and 1 gram suspended in olive oil, were injected subcutaneously. Subsequently the animal received 1 gram suspended in olive oil daily for five days. Food was not administered in this period. The glucose-nitrogen ratio was determined in order to demonstrate whether or not the dogs were in the diabetic state before beginning the experiment. In one experiment epinephrine was administered as a measure of depleting glycogen, but this was found to be superfluous because the fasting and ether anesthesia were found sufficient to reduce the glycogen in the muscle and liver to a satisfactorily low value. The specimens were removed either with the animal under the influence of ether anesthesia or after the animal had been killed. Control specimens were obtained in all cases preliminary to the injections of glucose. In the case of the skeletal muscles, control specimens were taken from corresponding muscles of opposite sides of the body. That such muscles can be used as controls had been amply confirmed. The determinations of glycogen were made according to a modification of Pflüger's method and the usual precautions in making determinations of glycogen in tissue were rigidly adhered to. A representative experiment follows.

Experiment 1. February 11, 1931. The dog used in this experiment weighed 10.8 kgm. After phlorhizination the first set of specimens of muscle and liver was removed in the usual manner, under ether anesthesia, and employing sterile technic; the second set was removed immediately after the animal had been killed. In the interval of twenty hours between taking of specimens, 1 gram of glucose for each kilogram of body weight each hour was injected intravenously. The glucose-nitrogen ratio before beginning the experiment was 3.62 to 1. The values for glycogen are given in table 1.

COMMENT. Much of the work that has been done on the problem of glycogenesis in phlorhizinized animals is open to criticism in that the experiments have not been adequately controlled. Many investigators have used one series of animals as controls and another series for the actual procedure in question. As is evident from a study of the values for glycogen in the various experiments, different animals of the same species vary widely in glycogen content of both liver and skeletal muscle. Not only do different animals exhibit this variation, but different muscles of the same animal differ widely in glycogen content. Curiously, corresponding muscles of opposite sides of the body of the normal, intact animal conform very closely in the amount of glycogen contained therein. For con-

TABLE 1

Values for glycogen in experiment 1

TISSUE	TIME	DATE	PER CENT GLYCOGEN
	a.m.		
Liver	9:00	Feb. 11	0.123
	5:00	Feb. 12	0.750
Left quadriceps muscle	9:03	Feb. 11	0.080
Right quadriceps muscle	5:06	Feb. 12	0.536
Left gracilis muscle	9:04	Feb. 11	0.066
Right gracilis muscle	5:07	Feb. 12	0.354
Left adductor muscle	9:05	Feb. 11	0.154
Right adductor muscle	5:08	Feb. 12	0.756
Left sartorius muscle	9:02	Feb. 11	0.095
Right sartorius muscle	5:07	Feb. 12	0.433

trol material, either the corresponding muscle, or part of the same muscle must be used. In the latter case, care must be exercised to preserve the blood supply. In such a case the distal part of the muscle is used as the first specimen and the proximal part as the final specimen.

In all experiments there was appreciable increase in glycogen content of the liver subsequent to administration of glucose, whereas in the skeletal muscles the glycogenesis was less marked. In five of the seven experiments, however, there was definite evidence of formation of glycogen in muscle.

It is evident that glycogenesis proceeds at a more rapid rate in the liver than in skeletal muscle of the phlorhizinized dog, but as compared with control experiments considerably less glycogen is formed in both the

liver and skeletal muscles of the phlorhizinized dog than in the normal animal under similar conditions.

Although this work cannot answer the question as to whether phlorhizin affects only the renal tissues, or whether it effects a general change in the process of metabolism of carbohydrate, nevertheless it does show that the totally phlorhizinized organism is capable of formation of glycogen.

SUMMARY

A series of seven experiments was performed in which phlorhizinized dogs received intravenous injections of glucose over varying intervals of time. Determinations of glycogen were made on both liver and skeletal muscle before and after injections of glucose. In all cases there was a significant increase in liver glycogen, whereas in five experiments the glycogen content of the skeletal muscle was also definitely increased.

From the data herewith presented it is evident that the totally phlorhizinized dog is capable of glycogenesis in both liver and skeletal muscle.

BIBLIOGRAPHY

Cori, C. F. 1925. Journ. Pharm. Exper. Therap., xxv, 1.
Csonka, F. A. 1916. Journ. Biol. Chem., xxvi, 93.
Junkersdorf, P. 1922. Pflüger's Arch., cxcvii, 500.
Nash, T. P., Jr. 1929. Journ. Biol. Chem., lxxxiii, 139.
Nash, T. P., Jr. and S. R. Benedict. 1923. Journ. Biol. Chem., lv, 757.
Palmer, W. W. 1917. Journ. Biol. Chem., xxx, 79.
Paulesco, N. and C. Michailesco. 1920. Compt. rend. Soc. de biol., lxxxiii, 566.
Ringer, A. I., H. Dubin and F. H. Frankel. 1921–1922. Proc. Soc. Exper. Biol. and Med., xix, 92.
Sato, K. 1923. Tohoku Journ. Exper. Med., iv, 347.
Török, P. 1924. Pflüger's Arch., cciv, 127.

THE EXCRETION OF URINE IN THE DOG

IV. The Effect of Maintenance Diet, Feeding, etc., Upon the Quantity of Glomerular Filtrate

JAMES A. SHANNON, NORMAN JOLLIFFE AND HOMER W. SMITH

From the Department of Physiology, University and Bellevue Hospital Medical College, New York City

Received for publication May 28, 1932

This paper deals primarily with the immediate effects of feeding, and of maintenance diets, upon the glomerular clearance in a single dog, using the excretion of xylose or sucrose as a means of determining the glomerular clearance as described by Jolliffe, Shannon and Smith (1932). (By glomerular clearance we refer to the volume of glomerular filtrate, as measured by the excretion of xylose or sucrose, in cubic centimeters of filtrate per square meter of body surface per minute.) The data are confined to experiments in which the rate of urine flow is above the augmentation limit for urea (Jolliffe and Smith, 1931a, b).

Jolliffe and Smith (1931a) have observed that the post-absorptive urea clearance in dogs is profoundly modified by the maintenance diet; when dog 18 was placed upon a daily ration of cracker meal, 100 grams, sucrose, 30 grams, and lard, 30 grams, the urea clearance fell within a few days from a range of 60–70 to 30, rising again (though not invariably) to 70 or more when the meat diet was resumed. In the following experiments the post-absorptive glomerular clearance in a single female collie (no. 36) has been followed first on a cracker meal diet and then on a meat diet, and observations have been made upon the immediate effects (post-prandial) of eating meat.

The observations begin with experiment 106, at which time the dog had been on a cracker meal diet for 22 days, and the post-absorptive glomerular clearance ranged somewhat below 50, varying slightly, as it usually does, from hour to hour. After a week more on the cracker meal diet the post-absorptive glomerular clearance was close to 40 (expt. 107), falling to 37 in the fifth half-hourly period of observation. The dog was then fed raw beef, and the glomerular clearance began to rise and at the end of five hours reached 74.8, which is an increase of about 100 per cent. The dog was continued on a meat diet for four days (expt. 110) when the post-absorptive glomerular clearance was low again (43.5–51.0); after a meat meal it rose to 75.0.

After two days additional on meat (expt. 111) the post-absorptive glo-

merular clearance ranged from 80.5 to 64.5; after a meat meal it rose to 105.0. Two days additional on meat (expt. 112) produced a post-absorptive clearance of 83.8 rising to a maximum level of 135.0 after meat. Thus in 8 days on a meat diet the post-absorptive glomerular clearance was observed to vary from a minimum of 37.1 to a maximum 83.8, and the post-prandial glomerular clearance was observed to reach a maximum of 135.0. This represents an increase of well over 300 per cent, most of which, we believe, represents a functional variation in glomerular activity.

Three days later, or after a total of 11 days on a meat diet, and after the dog had been fed meat twice on the previous day, the post-absorptive glomerular clearance ranged from 105 to 91, which contrasts with the lower and corresponding level in a single period of 83.8 three days before; after a second administration of xylose and water (but without meat) the clearance fell to 85. (This fall we are inclined to attribute to the additional diuresis resulting from the second administration of xylose and water, because we have observed that in general the glomerular clearance tends to fall during prolonged diuresis when the clearance is not at a basal level.)

The dog was then retired to a mixed diet (predominantly meat) for 20 days when she was again examined to determine the glomerular clearance at low rates of urine flow. Sucrose instead of xylose was used to measure the glomerular clearance in subsequent experiments because it exerts less osmotic pressure in the urine, and hence less diuretic action, at concentrations in the plasma suitable for quantitative determination. The glomerular clearance after a meal in experiment 122 (table 2) ranged from 112 to 138. Seven days later, after 4 days of fasting and 2 days on the cracker meal diet, the post-absorptive glomerular clearance ranged from 80 to 93 (expt. 125); and the next day from 66 to 79 (expt. 126).

The dog was then retired on a mixed (predominantly meat) diet again for a month and finally put upon a cracker meal diet for two weeks. She was given no water for the last three days. The post-absorptive glomerular clearance was observed to range from 38.5 to 52.8 (expt. 137) as when first examined. After three weeks more on the cracker meal diet the glomerular clearance as determined with xylose instead of sucrose ranged from 45 to 59 (expt. 144, table 1). After one week on a meat diet (expt. 147, which is not reported in full here) the post-prandial clearance rose to 106.5, an increase of 235 per cent.

In experiment 151 we have examined the post-prandial effects of eating cracker meal, butter and sugar, in another dog. The glomerular clearance during the pre-prandial period was about 72.0; it remained unchanged immediately after eating, but 5 hours later rose during a single, terminal period to 78.8. This figure may not have been the maximum, but when this result is compared with our previous experiments it appears that the

above food mixture has only a relatively slight action upon glomerular activity. Meat under these same conditions might be expected to raise the glomerular clearance above 100 (cf. expts. 112 and 113). It must be noted, however, that the above food mixture contains nearly 10 grams of protein which itself may account for the observed increase in glomerular activity.

MacKay, MacKay and Addis (1928) and MacKay and MacKay (1931) have shown that protein feeding leads to hypertrophy of the kidneys in the growing rat, and MacKay (1932) implies that the changes in the urea clearance of the dog on mixed as compared to cracker meal diets (Jolliffe and Smith, 1931a, b) are attributable in part to renal hypertrophy. We do not believe that our present results can be wholly explained by hypertrophy; we recognize that it is quite probable that protein feeding in the dog will lead to hypertrophy of the kidneys, as in the rat, but the changes in glomerular clearance which we have described are in adult dogs, and are too rapid and too reversible to be wholly due to hypertrophy. The glomerular clearance was increased from 37.1 to 74.8 in 5 hours (expt. 107); and four days later from 43.3 to 75.2 in the same period of time (expt. 110); after experiment 122 in which the post-absorptive glomerular clearance ranged from 112.0 to 138.3, it fell in eight days of fasting to a low value of 66 (expt. 126). We cannot exclude some hypertrophy and atrophy in our experiments, but certainly we cannot account for such large changes in such a short period of time exclusively on this basis.

Nevertheless this phenomenon is a peculiarly erratic one; the effect of meat tends to persist for some hours after meat feeding so that the effects of a continuous meat diet are cumulative; while on the other hand, the glomerular clearance when elevated by meat is very irregular and tends to drop abruptly to intermediate levels, particularly after prolonged diuresis. This instability at high levels confirms the opinion of Jolliffe and Smith (1931b) that a more uniform clearance may be obtained at low levels (i.e., on a cracker meal diet). Our experience has been that animals reduced to the lower level of glomerular clearance will show (particularly during and after diuresis) an astonishing constancy in behavior, whereas little uniformity can be expected at the elevated glomerular clearance levels observed on a mixed or a meat diet.

The effect of diet upon the $\frac{\textit{urea clearance}}{\textit{glomerular clearance}}$ *ratio.* It has been our experience so far that the ratio of the urea clearance to the glomerular clearance may vary considerably in different dogs, though we are not prepared to discuss this point further at this time. We have, however, followed the urea clearance on dog 36 throughout the above experiments because we felt that valid conclusions on the effect of diet could be drawn, at least at the present time, only in this way.

It will be noted that a change of $\pm$ 5.0 in empirical urea and xylose clear-

ances of 70 and 100, respectively, produces a change in the ratio of these clearances from 0.62 to 0.74. We believe that this variation is perhaps the magnitude which should be allowed in the above observations for experimental errors (analytical, failure to obtain representative blood or urine samples, or failure to obtain sufficiently flat blood plateaus of sugar, etc.) and consequently a difference in the urea:sugar ratio of ± 0.05 or less is hardly significant.

With this fact in mind it would appear that the urea:sugar ratio is not significantly affected by changing from a cracker meal diet to a meat diet, but remains between 0.65 and 0.75 (see expt. 106 as compared with expts. 110, 111, and 112); nor is this ratio significantly affected by the ingestion and metabolism of meat (expt. 107 and 110).

The effect of the rate of urine flow and of the urea concentration on the urea clearance: glomerular clearance ratio. Jolliffe and Smith (1931a, b) observed an *augmentation limit* for urea (cf. Austin, Stillman and Van Slyke, 1921) at a urine flow of about 0.2 cc. per minute in dogs maintained on a cracker meal diet, and at about 0.4 cc. in dogs maintained on a mixed diet. We cannot discuss at this time the glomerular clearance at urine flows below the augmentation limit, but we must note that at urine flows above 1.0 cc. per minute the rate of urine flow has no effect upon the urea:sugar ratio (expts. 106, 107, 110, 111 and 112) although the concentration of urea in the urine varies from 60 to 2375 mgm. per cent.

In the above experiments the concentration of urea in the plasma varied from 10 to 80 mgm. per cent with no effect upon the urea:sugar ratio.

In experiment 145 (table 1) urea was administered by stomach after the glomerular clearance had been reduced to a low level by maintenance on a cracker meal diet. In three control periods the urea:sugar ratio averaged 0.681; after the urea was administered, this ratio averaged 0.706. Thus no significant change in the urea:glomerular ratio occurred although the urine urea increased from 211 to 3330 mgm. per cent and the plasma urea from 16.4 to 209.2 mgm. per cent.

Thus, under the conditions described above, the urea:sugar ratio is independent of rate of urine flow, blood urea and urine urea concentration. This fact must ultimately be taken into account in considering the question of why the urea clearance is less than the glomerular clearance.

On the other hand, at rates of urine flow below 1.0 cc. per minute there is a significant tendency for the urea:sugar ratio to fall, as shown in experiment 144 with xylose and experiments 122, 125, 126 and 137 with sucrose. This fact suggests the possible passive reabsorption of urea, as was first argued by Rehberg (1926) from a comparison of the rates of excretion of urea and creatinine. But since the present data are not wholly suitable for the examination of this difficult question and since we believe that the problem is open to more direct experimental investigation, we will not dis-

TABLE 1

Effect of diet upon glomerular clearance

(All experiments except nos. 145 and 151 on dog 36)

PERIOD	TOTAL CONCURRENT TIME	URINE VOLUME PER MINUTE, V	UREA		XYLOSE		CM. = $\frac{UV}{P}$ / S.A.		CM. UREA / CM. XYLOSE
			Plasma	Urine	Plasma	Urine	Urea	Xylose	
			Experiment 106. December 23						
		cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*			
1	22	6.09	14.2	57.6	195	1,115	34.3	48.4	0.708
2	45	6.47	14.0	54.5	210	1,186	35.0	50.8	0.689
3	74	5.03	13.5	61.6	234	1,565	31.9	46.7	0.683
4	106	5.79	13.1	55.6	254	1,536	34.1	48.7	0.700
5	136	4.80	12.8	62.9	270	1,866	32.7	46.1	0.709
6	154	4.27	12.6	62.5	286	2,048	29.3	42.5	0.690
	(203)	† See note A. Xylose given *per os*							
7	240	3.22	11.5	99.7	333	3,895	38.8	52.4	0.740
8	270	2.50	11.1	94.4	338	4,000	29.6	41.1	0.720
9	318	3.15	10.7	100.4	336	4,080	41.0	53.1	0.772
	(348)	† See note B. Xylose given *per os*							
10	373	2.24	10.2	104.3	352	4,730	31.8	41.8	0.761
			Experiment 107. December 30						
1	31	1.90	11.8	135.1	194	3,380	30.4	46.0	0.661
2	60	2.58	11.8	90.0	224	2,642	27.3	42.3	0.646
3	91	3.61	11.8	70.0	255	2,222	29.7	43.7	0.680
4	122	4.00	11.9	58.0	290	2,000	27.1	38.3	0.708
5	155	3.64	11.6	62.4	302	2,220	27.2	37.1	0.733
	(193)	† See note A. Fed meat							
6	211	1.86	12.5	142.7	258	4,350	29.5	43.6	0.677
7	230	1.76	13.5	207.7	228	4,540	37.6	48.7	0.772
8	249	1.68	16.0	293.0	195	4,200	42.7	50.3	0.849
9	266	1.35	20.0	405.0	152	4,120	38.0	50.8	0.748
10	292	1.19	22.4	506.0	132	4,000	37.4	50.1	0.747
11	321	1.24	24.4	616.0	122	3,970	43.5	56.0	0.776
	(357)	† See note B. Xylose given *per os*							
12	394	1.57	25.4	528.0	158	4,700	45.4	64.9	0.700
13	424	1.93	25.3	473.0	194	4,760	50.1	65.8	0.761
14	461	2.06	25.2	441.0	200	4,880	50.1	69.8	0.718
15	493	2.03	25.1	483.0	180	4,770	54.4	74.8	0.727
			Experiment 110. January 4						
1	30	1.84	18.4	229	210	3,570	31.8	43.5	0.731
2	59	2.93	18.4	148	234	2,748	32.4	47.8	0.678
3	90	3.48	18.4	128	258	2,725	33.6	51.0	0.659
	(339)	† See note A. Fed meat							

TABLE 1—*Continued*

PERIOD	TOTAL CONCURRENT TIME	URINE VOLUME PER MINUTE, V	UREA		XYLOSE		CM. = $\frac{UV}{P} / S.A.$		CM. UREA / CM. XYLOSE
			Plasma	Urine	Plasma	Urine	Urea	Xylose	
Experiment 110. January 4—*Concluded*									
		cc.	*mgm. per per cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*			
4	372	2.09	26.7	512.	203	5,240	55.7	75.0	0.743
5	402	2.03	27.0	510.	201	5,280	53.2	73.8	0.722
6	443	2.22	27.6	513.	200	4,870	57.3	75.0	0.764
Experiment 111. January 6									
1	28	4.14	36.4	428	184	2,580	67.6	80.5	0.840
2	59	4.29	33.5	289	221	2,688	51.4	72.4	0.710
3	92	4.88	30.6	255	259	2,465	56.4	64.5	0.874
	(344)	† See note A. Fed meat							
4	376	2.38	53.2	1,230	173	5,120	76.4	97.8	0.781
5	407	2.55	56.0	1,157	168	5,000	73.2	105.5	0.684
6	440	2.57	58.9	1,326	162	4,760	80.3	104.8	0.766
Experiment 112. January 8									
1	32	2.45	54.1	1,087	182	4,480	68.5	83.8	0.818
	(299)	† See note A. Fed meat							
2	331	2.31	63.5	1,600	164	5,265	80.8	103.0	0.784
3	360	2.89	66.7	1,385	155	4,875	83.3	126.2	0.660
4	395	2.95	70.0	1,470	146	4,360	86.1	122.4	0.704
5	422	2.70	75.0	1,765	126	4,540	88.0	135.0	0.652
6	454	2.00	80.7	2,375	105	4,290	81.8	113.5	0.721
Experiment 113. January 11									
1	29	1.02	81.4	3,950	105	7,780	68.8	105.0	0.656
2	63	1.06	79.7	3,195	113	7,740	59.0	100.8	0.584
3	92	1.02	78.1	3,190	121	7,780	57.8	91.1	0.634
	(162)	† See note A. Xylose given *per os*							
4	190	2.92	66.7	1,075	259	5,480	65.3	85.8	0.752
5	213	2.78	66.0	1,068	245	5,350	62.5	84.3	0.742
6	235	2.96	65.2	1,094	231	5,160	68.9	91.8	0.750
Experiment 144. April 18									
1	31	1.355	14.5	280.5	140	4,180	36.4	56.2	0.648
2	64	1.635	14.2	239.5	148	3,850	38.3	59.1	0.648
3	94	1.600	13.9	239.5	146	3,850	38.3	58.6	0.654
4	151	1.260	13.5	271.2	122	3,500	35.2	50.2	0.702
5	220	0.696	12.8	425.5	62*	2,990	32.1	46.6	0.689

TABLE 1—*Concluded*

PERIOD	TOTAL CONCURRENT TIME	URINE VOLUME PER MINUTE, V	UREA		XYLOSE		CM. = $\frac{UV}{P}$ / S.A.		CM. UREA / CM. XYLOSE
			Plasma	Urine	Plasma	Urine	Urea	Xylose	
Experiment 144. April 18—*Concluded*									
		cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*			
6	262	0.487	12.3	559.0	30*	2,428	30.7	54.7	0.561
7	280	0.333	12.0	660.0	22*	2,169	23.1	45.6	0.506
Experiment 145. Dog 43									
1	30	1.66	16.8	241	172	3,520	33.1	47.2	0.702
2	60	2.00	16.6	211	191	3,595	35.3	52.3	0.675
3	92	1.72	16.4	211	200	3,910	30.7	46.8	0.658
	(186)	See note A. Xylose and urea *per os* and urea subcutaneously							
4	225	3.25	189.3	1,791	177	2,292	42.7	58.5	0.730
5	285	2.62	209.2	2,122	165	2,325	36.9	51.2	0.720
6	345	1.58	188.0	3,330	82	2,100	38.9	56.2	0.692
Experiment 151. Dog 30									
1	22	6.90	5.7	32.8	109	979	46.2	72.0	0.642
2	44	5.10	5.7	44.2	109	1,325	46.0	72.1	0.638
3	67	3.52	5.8	63.9	111	1,830	45.1	67.4	0.669
		See note A. Fed cracker-meal, butter and sugar							
4	91	3.37	6.1	75.3	110	1,910	48.4	68.0	0.712
5	115	2.83	6.4	92.0	98	2,220	47.3	74.6	0.632
	(314)	† See note B. Xylose given *per os*							
6	329	5.47	5.4	43.2	89	1,035	50.9	73.0	0.698
7	348	5.36	5.2	43.6	93	1,110	52.2	74.4	0.702
8	359	3.73	5.2	67.0	99	1,800	55.9	78.8	0.710

* Determined by adding 100 mgm. per cent glucose to blood filtrate after yeast extraction. Under these conditions the normal blood non-fermentable blank is about 2 mgm. per cent.

† Urine from wash-out period discarded.

cuss it at this time. Our observations that feeding meat increases the rate of excretion of urea relative to the blood urea confirms Addis and Drury (1923) who observed a similar result in man after feeding milk, a mixed meal, etc.

Discussion. The most interesting aspect of these experiments relates to the effect of diet upon the glomerular clearance.

That the glomerular clearance in animals other than the dog is neither constant nor maximal is established by several lines of evidence.

In his Harvey Lecture of 1920–21, Richards (1922) described experiments made with Plant (1917), showing that in the perfused mammalian kidney

small doses of adrenalin have a vasoconstrictor effect (raising the perfusion pressure) though paradoxically causing the kidney to swell. This he explained by the assumption that adrenalin constricts the efferent arterioles of the glomeruli and causes swelling and increased pressure within the glomerular capsule. This increased pressure leads to increased filtration and increased urine formation in spite of the reduced blood flow through the kidney as a whole. Richards also described experiments made with Schmidt on the transilluminated frog's kidney in which alternation of glomerular activity was observed in the living animal, some glomeruli showing rapid blood flow and others a sluggish flow as though the efferent arteriole were greatly constricted. These observations were extended in subsequent communications (Richards and Plant, 1922a, b; Richards and Schmidt, 1924). The number of active glomeruli may be increased by vaso-dilator agencies, etc. (section of sympathetics, injection of NaCl solution, glucose, urea, caffeine and pituitrin in small doses) and decreased by vaso-constrictor agencies (afferent nerve stimulation, hemorrhage, injection of adrenalin or pituitrin in large amounts). Further evidence of the constriction of the efferent arterioles in the frog glomerulus as induced by barium was presented by Mendenhall, Taylor and Richards (1924), and as induced by adrenalin, by Richards, Barnwell and Bradley (1927). White (1930) has more recently observed changes in the blood flow of the transilluminated kidney of *Necturus*, when the glomerular capillaries became congested and dilated. The efferent arteriole was observed to be noticeably constricted.

Bieter (1929) has confirmed the intermittent blood flow in the transilluminated frog's kidney as described by Richards and Schmidt, and the rôle of the sympathetics in maintaining this intermittency. Bieter has also called attention to the appearance in some glomeruli in the frog of a short capillary which connects the afferent with the efferent arteriole, thus affording a "shunt" which permits a rapid blood flow through the glomerulus, while circumventing any great filtration into the capsule. This observation is particularly interesting in that it affords an anatomical basis for glomerular inactivity while insuring continued circulation to the tubule through the efferent arteriole.

In mammals the first recorded evidence of intermittent glomerular function, apart from Richards and Plants' experiments with the perfused kidney, were afforded by Khanolkar (1922) who tried to demonstrate this intermittency by injection methods. After trying a variety of injection materials Khanolkar used carmine and hemoglobin; particularly with the latter he found in frozen sections of injected rabbit's kidney numerous glomeruli showing hemoglobin deposits. He concluded that not all the glomeruli are active at one time and that diuresis (saline plus caffeine) increased the proportion of active units. (The use of hemoglobin is open to

TABLE 2

Effect of diet upon glomerular clearance

(All experiments on dog 36)

PERIOD	TOTAL CONCURRENT TIME	URINE VOLUME PER MINUTE, V	UREA		SUCROSE		$C_M = \frac{UV}{P} / S.A.$		$\frac{C_M \text{ UREA}}{C_M \text{ SUCROSE}}$
			Plasma	Urine	Plasma	Urine	Urea	Sucrose	
			Experiment 122. February 1						
		cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*			
1	30	0.917	75.7	4,340	118.8	10,920	73.0	117.1	0.623
2	58	0.750	75.4	4,740	112.8	12,220	65.5	113.0	0.580
3	91	0.666	72.8	4,990	110.1	13,360	63.4	112.2	0.565
4	119	0.732	71.6	5,030	98.1	12,890	71.4	133.5	0.535
5	151	0.937	67.4	4,110	119.1	12,650	79.3	138.1	0.574
6	181	0.800	64.9	4,635	104.0	12,650	79.3	135.2	0.586
7	205	0.771	64.5	4,545	92.0	11,510	75.5	134.0	0.563
8	230	0.640	63.9	5,090	78.5	10,870	70.8	123.1	0.574
			Experiment 125. February 8						
1	32	0.706	7.5	338	171.8	14,000	44.2	80.0	0.552
2	62	0.780	7.2	298	160.1	12,733	44.9	86.1	0.522
3	125	0.627	7.2	365	140.3	13,025	44.2	80.8	0.547
4	157	0.484	7.2	492	97.0	13,390	45.9	92.8	0.495
5	192	0.365	7.2	643	69.3	12,350	45.3	90.4	0.501
6	232	0.247	7.2	864	48.0	11,145	41.1	79.6	0.516
			Experiment 126. February 9						
1	40	0.877	8.3	287	254.8	14,500	42.1	69.4	0.606
2	80	0.635	8.3	345	180.2	14,350	36.7	70.3	0.522
3	120	0.378	9.0	530	121.7	15,325	30.9	66.1	0.467
4	160	0.300	9.1	728	74.0	14,000	33.3	78.8	0.423
5	199	0.202	9.2	1,027	44.7	12,000	31.3	75.3	0.416
			Experiment 137. March 29						
1	60	0.463	14.4	744	135.4	9,575	33.2	45.5	0.746
2	114	0.363	13.8	752	101.9	9,795	27.5	48.5	0.567
3	174	0.325	13.4	892	68.4	8,010	30.0	52.8	0.566
4	237	0.232	13.3	1,141	42.9	5,130	27.6	38.5	0.717

the objection that it produces profound changes in kidney volume, and probably disturbances in glomerular activity—a danger which attends all injection methods. Cf. Reid, 1929 and Mason and Mann, 1931.) Hayman and Starr (1925), from injection experiments with Janus green, concluded that the number of open glomeruli in the rabbit's kidney varies widely under spontaneous and experimental conditions. The proportion

of active glomeruli is increased by caffeine and saline and decreased by adrenalin and CO_2 inhalation.

Sheehan (1931) has observed that after injection of dyes into rabbits some tubules may be stained while others are not, which fact indicates intermittency of activity.

Bensley (1929) has examined the efferent arterioles in mammals and decribes a network of cells (pericytes) investing them which resemble Rouget cells somewhat in the smaller animals, but which approach true smooth muscle cells in structure in the case of man. He considers these pericytes to be a mechanism admirably adapted for the intrinsic control of glomerular activity and pressure.

Winton (1931a, b), working with the isolated dog kidney, has reached conclusions in line with the observations which Richards has made upon the frog; the quantity of glomerular filtrate can be increased by dilatation of the afferent arterioles and constriction of the efferent arterioles (adrenalin in small doses, etc.) while constriction of the afferent arterioles (larger doses of adrenalin) reduces the quantity of glomerular filtrate. According to Winton, caffeine increases the glomerular pressure and blood flow by dilatation of the afferent arteriole; low concentrations of adrenalin have the same effect on the glomerular pressure but reduce the blood flow by constricting the efferent arteriole; higher concentrations of adrenalin reduce glomerular pressure and blood flow by constricting the afferent arteriole. Pituitary extracts, according to Winton, reduce the urine flow by increasing the rate of reabsorption of water and not by a vasomotor action.

The above observations supplement our present findings that the glomerular activity varies over a wide range in the normal, intact, unanesthetized dog. There is nothing in our data on the effect of diet to indicate how these changes in glomerular activity are brought about in the kidney; i.e., whether by changes in effective glomerular filtration pressure or total filtering surface. The former might result from changes in efferent or afferent arterial tone, total blood flow through kidney or systemic arterial pressure, and the latter from changes in the total number of active glomerular capillaries, whether mediated by "shunts" or otherwise. (We know of no histological evidence which definitely precludes the existence of capillary shunts between the afferent and efferent arterioles in the mammal similar to those observed by Bieter in the frog.) Nor is there evidence here to enable us to decide whether the changes in glomerular activity are effected through some humoral factor associated with the high protein metabolism characteristic of a meat diet, or whether these changes are effected by way of the sympathetic nervous system. In addition, it is recognized that there may be changes in the protein content of the plasma brought about by diet, but these would be expected to act in the reverse

direction since the plasma protein should rise and tend to retard glomerular filtration on a high protein diet.

But it is possible to conclude from these data that there are important changes in the functional activity of the glomeruli associated with metabolism, and that these changes in functional activity tend to be cumulative so that the net result of a week's maintenance on a particular diet may be much more marked than the results of a single meal, even when the functional activity of the kidneys is observed in the standard, post-absorptive condition.

It would appear that a similar phenomenon occurs in man if we may make an approximation from the data on Deuel, Sandiford, Sandiford and Boothby (1928). During the period when Deuel was living on a low protein diet the blood ureas remained consistently at 14 mgm. per cent. Doctor Boothby has kindly furnished us with the urine volumes which enable us to calculate Deuel's urea clearance from the 24 hour data. The standard clearance $\left(\frac{U\sqrt{V}}{B}\right)$ was 53.4 at a urine flow of 0.475 cc. per minute on the first day on the low protein diet, and fell to 17.5 on the third day (urine flow, 1.21 cc. per minute) and to 9.6 on the 32nd day (urine flow, 0.347 cc. per minute). If the urea clearance parallels the glomerular clearance in man as closely as it has in the experiments reported here on the dog, these calculations would indicate that Deuel's glomerular filtrate was very significantly reduced by subsistence on a low protein diet.

SUMMARY

Observations on a single dog fed alternately on a cracker meal diet and a meat diet show that the glomerular clearance (xylose or sucrose clearance) can be increased nearly 3½-fold by feeding meat, i.e., from 40 to 135 cc. per square meter of body surface per minute.

The glomerular clearance can be increased two-fold in the course of 4 or 5 hours after a single meal of raw beef but the maximal effect is obtained after feeding meat when meat is also used as a maintenance diet. The glomerular clearance tends to persist at an elevated level for some hours or days after meat feeding, so that the effect of a meat diet is cumulative.

Evidence is presented in favor of the view that this elevation in the glomerular clearance is largely due to increased glomerular filtration, rather than to hypertrophy of the kidneys.

While on a meat diet the glomerular clearance tends to be erratic and may fall abruptly to intermediate levels, which again argues against this elevation being due to hypertrophy of renal tissue.

The urea clearance is invariably less than the glomerular clearance. There is no change in the urea: glomerular clearance ratio with changes in urine flow above 1.0 cc. per minute, and with changes in plasma urea from

16.4 to 209.2 mgm. per cent, or with changes in urine urea from 211 to 3330 mgm. per cent. Beyond noting these facts, no comment is made on this point.

Protocols Referring to Data in Tables Showing Effect of Diet Upon Glomerular Clearance. Dog 36: Weight throughout = 15 kgm., surface area = 0.72 sq. m. Female collie. *Experiment 106.* . . . . December 23. Cracker meal diet 22 days, water *ad. lib.* Given 600 cc. water by stomach at 8:30 a.m. Forty-five grams xylose in 600 cc. water by stomach at 9:40 a.m. Fifteen grams xylose in 225 cc. water by stomach at 10:15 a.m. Period 1 began at 10:50 a.m. Blood drawn at 3, 74, 156, 205, 272, 320 and 350 minutes, and urea and xylose interpolated for middle of each urine period. Note A: 30 grams xylose in 225 cc. water by stomach at end of period. Note B: 45 grams xylose in 90 cc. water at end of period 9. At beginnnng of period 9 dog saw meat being prepared for other dogs.

Experiment 107. Dog 36. December 30. Cracker meal diet 29 days. Water *ad lib.* Given 45 grams xylose in 450 cc. water by stomach at 7:56 a.m., 15 grams xylose in 150 cc. water by stomach at 8:30 a.m. Period 1 began at 9:00 a.m. Blood drawn at 15, 92, 157, 212, 268, 325, 375, 426 and 495 minutes, and urea and xylose interpolated for middle of each urine period. Note A: at end of period 5 dog ate 900 grams raw beef in which were mixed 45 grams xylose. Note B: 30 grams xylose in 300 cc. water by stomach at end of period 11.

Experiment 110. Dog 36. January 4. Meat diet since December 30. There is a possibility dog did not eat meat the day before this experiment. Given 45 grams xylose in 600 cc. water by stomach at 8:15 a.m., 15 grams xylose in 225 cc. water by stomach at 8:45 a.m. Period 1 began at 9:15 a.m. Blood drawn at 15, 75, 355 and 421 minutes and urea and xylose interpolated. Note A: Dog ate 900 grams of raw beef at end of period 3. Forty-five grams xylose in 600 cc. water by stomach 84 minutes before start of period 4. Hematocrit blood 1 = 37.2 per cent; blood 4 = 36.5 per cent. Total plasma nitrogen blood 4 = 1071 mgm. per cent.

Experiment 111. Dog 36. January 6. Meat diet since December 30. Water *ad lib.* Given 45 grams xylose in 600 cc. water by stomach at 8:10 a.m., 15 grams xylose in 225 cc. water by stomach at 8:40 a.m. Period 1 began at 9:17 a.m. Blood drawn at 13, 74, 359 and 419 minutes, and urea and xylose interpolated for middle of each urine period. Note A: Dog ate 900 grams raw beef at end of period 3. Forty-five grams xylose in 600 cc. water by stomach 75 minutes before beginning of period 4. Hematocrit, blood 4 = 30.0 per cent. Total plasma nitrogen blood 4 = 923 mgm. per cent.

Experiment 112. Dog 36. January 8. Meat diet since December 30. Dog ate 900 grams raw beef twice daily January 6 and 7. Forty-five grams xylose in 600 cc. water by stomach at 8:15 a.m. Period 1 began at 9:15 a.m. Note A: Dog ate 900 grams raw beef at end of period 1, 45 grams xylose in 600 cc. water by stomach 59 minutes before beginning of period 2. Blood drawn at 15, 315, 375 and 435 minutes and urea and xylose interpolated.

Experiment 113. Dog 36. January 11. Meat diet since December 30. No water allowed since 9:00 a.m. January 9. Dog ate 900 grams raw beef January 8, 900 grams January 9, 900 grams at 11:00 a.m. and 900 grams at 5 p.m. January 10. On January 11, 30 grams xylose in 400 cc. water injected subcutaneously at 8:15 a.m. Seven and one-half grams xylose in 100 cc. water injected subcutaneously at 8.45 a.m. Period 1 began at 9:00 a.m. Note A: At end of period 3, 30 grams xylose in 600 cc. water given by stomach. Thirty-seven minutes before beginning of period 4, 15 grams xylose in 225 cc. water given by stomach. Blood drawn at 15, 75, 177 and 224 minutes

and urea and xylose interpolated. Hematocrit blood 1 = 37.0 per cent; blood 2 = 39.0 per cent. Total plasma nitrogen, blood 1 = 984 mgm. per cent.

Experiment 122. Dog 36. February 1. Mixed diet since January 11. Dog ate 900 grams raw beef January 30 and 31. Water removed from cage 2 p.m. January 31. On February 1 fed 900 grams raw beef at 7 a.m. Drank 360 cc. water at 8:15 a.m. Ten and one-half grams sucrose in 150 cc. water injected subcutaneously at 8:25 a.m. and 4.5 grams sucrose in 50 cc. water injected subcutaneously at 9:00 a.m. Period 1 began at 9:31 a.m. All blood samples drawn at middle of urine periods. At 93 minutes gave 600 cc. water by stomach, and at 94 minutes 7.5 grams sucrose in 100 cc. water subcutaneously. Hematocrit, blood 8 = 40.2 per cent.

Experiment 125. Dog 36. February 8. Dog fasted February 2 to 6. Cracker meal diet February 6 to 7. No water since 5:00 p.m. February 6. On February 8, 19.5 grams sucrose in 180 cc. water injected subcutaneously. Period 1 began at 9:13 a.m. All blood samples drawn at middle of urine periods. Hematocrit, blood 7 = 35 per cent.

Experiment 126. Dog 36. February 9. Experiment 125 continued without water. Nineteen and one-half grams sucrose in 200 cc. water subcutaneously at 8:15 a.m. Period 1 began at 9:00 a.m. All blood samples drawn at middle of urine periods. Hematocrit, blood 7 = 30.5 per cent.

Experiment 137. Dog. 36. March 29. Mixed diet February 10-March 15. Cracker meal diet March 15–28. No water since 9:00 a.m. March 26. On March 29, 15 grams sucrose in 100 cc. water injected subcutaneously. Period 1 began at 10:03. All blood samples drawn at middle of urine periods.

Experiment 144. Dog 36. April 18. Cracker meal diet since March 15. No water since April 16. On April 18, 22.5 grams xylose in 90 cc. water by stomach at 8:15 a.m. and 7.5 grams xylose in 45 cc. water by stomach at 8:45 a.m. Period 1 began at 9:59 a.m. All blood samples drawn at middle of urine periods.

Experiment 145. Dog 43. Weight 19 kgm., S. A. 0.92 sq. m. April 20. Cracker meal diet since March 15. No water since 5:00 p.m., April 19. Twenty-eight and one-half grams xylose in 190 cc. water by stomach at 9:00 a.m., 9.5 grams xylose in 57 cc. water by stomach at 9:30 a.m. Period 1 began at 10:30 a.m. Note A: At end of period 3, 14.3 grams urea and 14.3 grams xylose in 76 cc. water by stomach and 14.3 grams urea in 100 cc. water subcutaneously. All blood samples drawn at middle of urine periods.

Experiment 151. Dog 30. Weight 20 kgm., S. A. 0.86 sq. m. May 11. Cracker meal diet since May 6. Thirty grams xylose in 800 cc. water by stomach at 9:30 a.m., 10 grams xylose in 400 cc. water by stomach at 10:05 a.m. Period 1 began at 11:04 a.m. Note A: At end of period 3 dog ate 100 grams cracker meal, 30 grams sucrose and 30 grams butter. Note B: 30 grams xylose in 800 cc. water by stomach at 205 minutes. Ten grams xylose in 400 cc. water by stomach at 235 minutes.

BIBLIOGRAPHY

ADDIS, T. AND D. R. DRURY. 1923. Journ. Biol. Chem., lv, 629.

AUSTIN, J. H., E. STILLMAN AND D. D. VAN SLYKE. 1921. Journ. Biol. Chem., xlvi, 91.

BENSLEY, R. D. 1929. Amer. Journ. Anat., xliv, 141.

BIETER, R. N. 1929. This Journal, xci, 436.

DEUEL, H. J., I. SANDIFORD, K. SANDIFORD AND W. M. BOOTHBY. 1928. Journ. Biol. Chem., lxxxvi, 391.

HAYMAN, J. M., JR. AND I. STARR. 1925. Journ. Exp. Med., xlii, 641.

JOLLIFFE, N. AND H. W. SMITH. 1931a. This Journal, xcviii, 572.
1931b. This Journal, xcix, 101.
JOLLIFFE, N., J. A. SHANNON AND H. W. SMITH. 1932. This Journal, c, 301.
KHANOLKAR, V. R. 1922. Journ. Path. and Bact., xxv, 414.
MACKAY, E. M. 1932. This Journal, c, 402.
MACKAY, E. M. AND L. L. MACKAY. 1931. Journ. Nutri., iii, 375.
MACKAY, E. M., L. I. MACKAY AND T. ADDIS. 1928. This Journal, lxxxvi, 459.
MASON, J. B. AND F. C. MANN. 1931. This Journal, xcviii, 181.
MENDENHALL, W. L., E. M. TAYLOR AND A. N. RICHARDS. 1924. This Journal, lxxi, 174.
REHBERG, P. B. 1926. Biochem. Journ., xx, 461.
REID, W. L. 1929. This Journal, xc, 168.
RICHARDS, A. N. 1920–21. Harvey Lectures, xvi, 163.
1922. Amer. Journ. Med. Sci., clxiii, 1.
RICHARDS, A. N., J. B. BARNWELL AND R. C. BRADLEY. 1927. This Journal, lxxix, 410.
RICHARDS, A. N. AND O. H. PLANT. 1917. This Journal, xlii, 592.
1922a. This Journal, lix, 184.
1922b. This Journal, lix, 191.
RICHARDS, A. N. AND C. F. SCHMIDT. 1924. This Journal, lxxi, 178.
SHEEHAN, H. L. 1931. Journ. Physiol., lxxii, 201.
WHITE, H. L. 1930. Proc. Soc. Exp. Biol. Med., xxvii, 613.
WINTON, F. R. 1931a. Journ. Physiol., lxxii, 361. lxxiii, 151.

THE EXCRETION OF URINE IN THE DOG

V. The Effects of Xylose and Sucrose Upon the Glomerular and Urea Clearances

NORMAN JOLLIFFE, JAMES A. SHANNON AND HOMER W. SMITH

From the Department of Physiology, University and Bellevue Hospital Medical College, New York City

Received for publication May 28, 1932

In view of the fact that the glomerular clearance is profoundly modified by diet and other physiologically active agents (cf. Shannon, Jolliffe and Smith, 1932) it is pertinent to inquire if xylose or sucrose themselves exert any action upon renal activity. It is obvious that this question can be answered only by reference to the rate of excretion of some substance other than xylose or sucrose, and at the present time urea is the only substance suitable for this purpose. We have shown that at rates of urine flow above 1 cc. per minute the ratio of the urea to the glomerular clearance was essentially constant for one dog (0.65–0.75), although the glomerular clearance was increased three and a half fold by feeding meat. In view of this fact we feel that if it could be shown that neither xylose nor sucrose exerted any significant action upon the urea clearance, this fact might be advanced as evidence that these sugars did not *per se* influence the glomerular clearance when administered to the normal dog. The point, we think, is singularly important because of the great variations in the glomerular clearance which we have observed in dogs to which these sugars have been administered for the purpose of measuring the glomerular filtrate.

As a preface to the investigation of this question it is necessary to examine certain conditions of experiments of this nature. A serious difficulty, and one to which reference is rarely made in studies of renal activity, is the observational error introduced by the dead space of the tubules, ureters and bladder; this error becomes particularly significant when the rate of urine flow is changing rapidly; at such times it may lead to entirely misleading results in the calculation of the glomerular clearance (or the clearance of urea or any other substance, for that matter). This dead space error can, of course, be avoided to a great extent by diuresis induced by the preliminary administration of large quantities of water (as in

the standard conditions of Addis (1922) and Taylor, Drury and Addis (1923)).[1]

But a second difficulty is the marked sensitivity of the glomeruli in the normal animal. A phenomenon of special interest here is that the administration of water (or salt solution) by stomach may lead to an increase in the glomerular clearance under conditions where there is no significant change in the rate of urine flow (and therefore no dead-space error), or when an appropriate correction for the dead-space is applied.

The first experiment which we wish to describe illustrates both of the above points well.

Experiment 103 (table 1) was performed upon a dog subsisting on a cracker meal diet and which has been used frequently in experiments of this kind and was therefore thoroughly accustomed to the routine. The urea clearance at natural urine flows (periods 1 and 2), which lay below the augmentation limit, was first determined early in the morning. Water was then given by stomach; as diuresis developed a urine containing concentrated urea from period 3 was swept out of the kidney by the less concentrated urine of period 4 which was now flowing at a greatly increased rate. The result of the dead-space error here is to produce an apparent urea clearance of 114, as compared with the probable clearance of 50 (the rate of urine flow was increasing from 0.094 to 0.856 cc. per minute). A similar effect is evident in period 5 in which the apparent clearance is 71.0, since the rate of urine flow is still increasing (0.856 to 4.66 cc. per minute). The effect of the dead-space can be illustrated by a simple calculation on period 5: during this period 200 mgm. of urea were excreted; if we assume that the dead-space is 8 cc. and deduct 87.5 mgm. (0.08×1094) for urea left from period 4 and add 6.8 mgm. (0.08×85.5) for urea properly belonging to period 6, we arrive at a true excretion for period 5 of 119.3 mgm. or a true urea clearance of 48.8 instead of 71.0. Thus the difference in the urea clearance in periods 5 and 6 appears to be largely an error which is due to a rapidly increasing rate of urine flow.

Dead-space error is not the only factor here, however, as is shown by the next two periods. After another dose of water by stomach the urea clearance increased from 49.2 (period 6) to 60.8 (period 7) although there was no change in urine flow in period 7 as compared with period 6. Apparently the administration of water in this case really increased the urea clearance. This phenomenon occurs again after the fourth dose of water (periods 8 and 9), yet when a correction is applied to period 9, on the basis

[1] In our previous experiments on diet, etc., dead-space error was avoided by the preliminary administration of water as shown in our protocols, and in our experiments comparing the excretion of xylose, glucose, sucrose and raffinose (Jolliffe, Shannon and Smith, 1932), apart from the water administered beforehand to induce diuresis, no water was administered by stomach during the experiments.

of an 8 cc. dead-space, this correction does not change the urea clearance from the observed figure. It may be noted that the urea clearance invariably falls during the second thirty-minute period after the administration of water, a fact which supports the idea that the clearance has previously been increased by the administration of water.

It is evident, however, that by the end of period 10 the urea clearance is fairly constant. At this time the same quantity of water was administered as before, but with a suitable quantity of xylose dissolved in it. The urea clearance increased very slightly, but no more than between periods 6 and 7 or between 8 and 9. At the end of period 12, water and xylose were again administered and produced the customary slight rise in the urea clearance.

By the end of period 14, it was possible to observe the glomerular clearance as measured by the excretion of xylose.

A large quantity of water with xylose now produced the usual slight rise in urea clearance (if corrected for an 8 cc. dead-space the urea clearance for period 15 would still be 55.0, as compared with 51.8 in the previous period); and what is more noteworthy, a corresponding rise in the glomerular clearance. Numerous experiments which will not be reported in detail here indicate that the increase in the urea clearance which results from the administration of water *per os* is due to a corresponding increase in the glomerular clearance.

The effect of the enteric administration of water (in the form of xylose solutions) on the glomerular clearance is also illustrated in experiments previously published by us (Shannon, Jolliffe and Smith, 1932); experiment 106, periods 6–7, 42.5 to 52.4; experiment 107, periods 11–12, 56.0 to 64.9; experiment 145, periods 3–4, 46.8 to 58.5. None of these instances can be explained on the basis of dead-space alone, and it appears from experiment 103 that the phenomenon is not due to the xylose since the administration of xylose solutions exerts no greater effect than does the administration of water only.

It appears from these observations that this water-effect represents an increase in the glomerular clearance which is followed more or less passively by the urea clearance. We have described the phenomenon here because of the necessity of considering it in relation to the subject matter of this paper, although we have no explanation to offer for it at this time.

The next experiment (exp. 89, table 1) concerns the effect of xylose when administered subcutaneously on the urea clearance. In this experiment the urea clearance was not observed immediately after the xylose injection; at the end of an hour and a half, however, when the glomerular clearance could be measured by the excretion of xylose the urea clearance was close to what it had been in the last of the control periods—the injection of xylose had had no effect upon it.

Experiment 120 (table 2) concerns the effect of sucrose injected subcutaneously on the glomerular clearance (xylose) and the urea clearance, as observed in a dog on a mixed diet. In this case the xylose was adminis-

TABLE 1

The effects of the administration of xylose on the urea clearance in dogs

PERIOD	TOTAL CONCURRENT TIME	URINE VOLUME	UREA		XYLOSE		CM. = $\frac{UV}{P}$ / S.A.		CM. UREA / CM. XYLOSE
			Plasma	Urine	Plasma	Urine	Urea	Xylose	
			Expt. 103. Dog 36						
	minutes	*cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*			
1	60	0.150	9.9	1,479			31.1		
2	95	0.072	10.7	2,370			22.1		
	40 cc. water per kgm. by stomach								
3	127	0.094	11.4	2,326			26.6		
4	158	0.856	11.4	1,094			114.1		
	15 cc. water per kgm. by stomach								
5	192	4.66	11.5	126.2			71.0		
6	220	4.89	11.5	85.5			49.2		
	15 cc. water per kgm. by stomach								
7	244	4.95	11.4	100.7			60.8		
8	283	4.69	11.2	94.4			54.9		
	15 cc. water per kgm. by stomach								
9	310	5.48	10.9	84.0			58.6		
10	341	4.39	10.7	93.4			53.2		
	15 cc. water and 2.5 grams xylose per kgm. by stomach								
11	372	1.45	10.5	295.0			56.6		
12	404	3.19	10.4	118.2			50.4		
	15 cc. water and 0.5 grams xylose per kgm. by stomach								
13	437	2.87	10.3	143.9	135.0	2,885	55.6	85.2	0.652
14	468	2.80	10.1	134.4	151.5	2,775	51.8	71.2	0.628
	40 cc. water and 0.5 gram xylose per kgm. by stomach								
15	500	4.19	9.8	96.8	157.7	2,082	57.5	76.8	0.748
16	532	5.78	9.5	64.4	159.6	1,500	54.4	75.4	0.721
			Expt. 89. Dog 30						
1	32	2.69	42.8	106			77.4		
2	62	5.49	38.3	445			74.2		
	(122)	See note A. Xylose subcutaneously							
3	152	1.20	34.8	1,730	82	5,870	69.4	100.0	0.694
4	182	1.03	31.2	1,892	83	7,060	72.6	102.0	0.711

tered by stomach beforehand with sufficient water to produce a prolonged diuresis, and the glomerular and urea clearances were observed without interruption after the injection of sucrose. Neither the urea nor the

glomerular clearance was affected by the sucrose beyond the small variations which we have observed to occur spontaneously. Experiments 89 and 120 show that xylose and sucrose do not affect the glomerular or the urea clearance when these are elevated by a meat diet, while experiment

TABLE 2

The effects of administering xylose and sucrose on the urea and glomerular clearances in dogs

PERIOD	TOTAL CONCURRENT TIME	URINE VOLUME	UREA		XYLOSE		SUCROSE		CM. = UV/P / S.A.			CM. UREA / CM. XYLOSE	CM. XYLOSE / CM. SUCROSE
			Plasma	Urine	Plasma	Urine	Plasma	Urine	Urea	Xylose	Sucrose		
	Expt. 120. Dog 20												
	minutes	*cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*					
1	83	2.75	63.7	1,682	82	2,732			95.5	120.5		0.792	
2	99	4.12	62.3	989	86	1,859			86.0	117.2		0.734	
3	120	4.19	61.3	1,047	106	2,200			94.1	114.5		0.821	
	See note A: Sucrose and more xylose subcutaneously												
4	179	5.29	57.5	746					90.2				
5	201	3.63	53.2	1,069	136	3,410	111	2,885	96.0	119.7	124.1	0.802	0.965
6	223	3.91	51.5	923	136	2,986	106	2,288	92.2	112.9	111.0	0.817	1.018
7	240	4.47	50.2	805	136	2,552	104	1,926	94.3	110.4	109.0	0.854	1.012
	Expt. 146. Dog 43												
1	41	1.12	20.4	471					28.1				
2	64	0.91	20.4	579					28.1				
3	84	0.70	19.7	720					27.8				
	See note A: xylose by stomach												
4	127	0.71	18.7	732					30.2				
5	148	1.10	18.0	405	140	4,285			26.9	36.5		0.737	
6	166	1.56	17.9	337	174	4,385			31.9	42.7		0.747	
7	185	1.48	17.6	292	189	4,235			26.7	36.1		0.740	
	See note B: sucrose subcutaneously												
8	214	1.66	17.3	260					27.1				
9	245	2.19	17.0	181	202	3,625			25.3	42.7			
10	265	2.05	17.3	188	212	3,400	208	3,270	24.4	35.8	35.0	0.681	1.023
11	304	2.20	17.6	195	219	3,625	246	4,080	26.5	39.6	39.7	0.669	0.998
12	324	2.10	17.9	229	178	3,355	256	4,850	29.2	43.0	43.3	0.679	0.994

103 shows the absence of any action by xylose at the reduced levels of glomerular activity which are characteristic of a cracker meal diet.

It seemed that the most exacting experiment for demonstrating the physiological indifference of xylose and sucrose would be one in which the

conditions were such as to give a reduced, steady glomerular clearance, and one in which the effect of water administration was eliminated as far as possible. To obtain these conditions, a dog was used which had been kept upon a cracker meal diet for 11 days and in which the clearance was known to be down to the typical, basal level. (Cf. expt. 146, table 2.) Ample water was administered to produce a copious diuresis; the water was given four hours beforehand so that the experiment could be conducted entirely in the post-diuretic period.

After three control periods, during which the urea clearance was observed to average 28.0, xylose in concentrated solution was administered by stomach. In the next 43 minute period the urea clearance rose to 30.2 (a negligible increase) while the average of four periods extending over an hour and a half was 28.9. Then sucrose and a small quantity of xylose were injected subcutaneously; the urea clearance remained essentially unchanged in the next half-hour period, and for the next two hours and a quarter averaged 26.5.

The glomerular clearance, as measured by the xylose excretion, in periods 5, 6 and 7 averaged 38.4; after sucrose, in periods 9, 10, 11 and 12, it averaged 40.3. The xylose:urea ratio changed from 0.74 before sucrose to 0.68 after sucrose, but since a ± 3.0 per cent error in the urea and xylose clearances at 30 and 40, respectively, produces a change in the ratio of these clearances of 0.09, the observed change of 0.06 cannot be considered to be particularly significant.

Thus, under the conditions of this experiment, the urea clearance remained fairly constant although sufficient xylose was administered by mouth, and sufficient sucrose parenterally, to permit the determination of the rate of glomerular filtration by either sugar, and in spite of the fact that the concentration of total sugar in the urine reached 8 per cent. Furthermore, the sucrose did not significantly affect the glomerular clearance as measured by xylose.[2]

The above observations were all made at moderate to high rates of urine flow, and therefore with correspondingly low concentrations of xylose and sucrose in the urine (4.4 and 3.6 per cent, respectively), and it cannot be argued from them that these sugars, if present in the urine in very high concentrations, would not modify the normal urea clearance. (Sucrose may be concentrated to 25 per cent in the dog.) We do not believe that this question can be answered by experiments of the type which we have used here, since below the augmentation limit the rate of excretion of urea is intimately related to the rate of urine flow (Austin, Stillman and Van Slyke, 1921).

[2] The correspondence between the xylose and sucrose clearances in experiments 120 and 146 confirms our previously recorded observations that these sugars are excreted, in simultaneous experiments, in an identically quantitative manner.

Our experiments are sufficient, however, to assure us that in using xylose and sucrose to measure the glomerular clearance we are not administering to the animal substances which modify either the state of activity of the glomeruli, or the urea clearance.

It is also appropriate to refer at this time to the fact that dog 36 (see Shannon, Jolliffe and Smith, 1932) received xylose and sucrose repeatedly over a period of four months without any evidence of impairment in renal function. The most significant criterion of this fact, we think, is the constancy of the urea : glomerular clearance ratio throughout the period. We emphasize this ratio because we feel that tubular injury (whether acute or chronic) will probably first reveal itself by an increased permeability and consequently an increased diffusion of urea back into the renal blood and lymph, as has been suggested by Rehberg (1926) and Holten and Rehberg (1931). The result of this diffusion will of course be to lower the urea : glomerular clearance ratio.

SUMMARY

It is pointed out that the administration of water by stomach may lead to an increase in the glomerular (and therefore the urea) clearance.

It is shown that in properly conducted experiments (and under conditions in which the urea clearance has been shown to parallel the glomerular clearance), the administration of xylose solutions by stomach or by subcutaneous injection does not significantly modify the urea clearance; and that the subcutaneous injection of sucrose solutions does not significantly modify either the xylose clearance or the urea clearance.

It is concluded that xylose and sucrose are physiologically inert, so far as renal function is concerned, if used in the manner described here, and may safely be used to measure the glomerular clearance without danger of perturbing effects upon the glomeruli, or upon the excretion of urea.

Protocols accompanying experiments in tables 1 and 2. Experiment 103. Dog 36. Weight 15 kgm., S. A. 0.72 sq. m. Cracker meal diet, 12 days. Water and xylose given by stomach tube as indicated in table. Blood drawn at 0, 98, 222, 374, 422, 457, 483 and 516 minutes. Plasma urea and xylose concentrations interpolated to middle of each urine period.

Experiment 89. Dog 30. Weight 15 kgm., S. A. 0.86 sq. m. Meat diet, one week. Eighteen grams xylose in 100 cc. water subcutaneously at 62 minutes and 4.5 grams xylose in 20 cc. water subcutaneously at 107 minutes. All blood samples drawn at middle of urine periods.

Experiment 120. Dog 20. Weight 18 kgm., S. A. 0.76 sq. m. Mixed diet 47 days. Fifty-four grams xylose in 720 cc. water by stomach at 8:15 a.m. and 9 grams xylose in 270 cc. water by stomach at 8:45 a.m. Period 1 began at 9:14 a.m. Note A.: 18 grams sucrose in 180 cc. water subcutaneously at 125 minutes and 18 grams xylose in 270 cc. water by stomach at 135 minutes. All blood samples drawn at middle of urine periods.

Experiment 146. Dog 43. Weight 19 kgm., S. A. 0.92 sq. m. Cracker meal diet 16 days. Seven hundred sixty cubic centimeters water by stomach at 8:15 a.m.

Bladder emptied and period 1 began at 12:15 p.m. Note A: 38 grams xylose in 38 cc. water by stomach at 86 minutes. Note B: 38 grams sucrose and 9 grams xylose in 150 cc. water subcutaneously at 189 minutes. All blood samples drawn in middle of collection periods.

BIBLIOGRAPHY

ADDIS, T. 1922. Arch. Int. Med., xxx, 378.
AUSTIN, J. H., E. STILLMAN AND D. D. VAN SLYKE. 1921. Journ. Biol. Chem., xlvi, 91.
HOLTEN, C. AND REHBERG, P. B. 1931. Acta. Med. Scand., lxxiv, 479.
JOLLIFFE, N., J. A. SHANNON AND H. W. SMITH. 1932. This Journal, c, 301.
REHBERG, P. B. 1926. Biochem. J., xx, 461.
SHANNON, J. A., N. JOLLIFFE AND H. W. SMITH. This Journal, in press.
TAYLOR, F. B., D. R. DRURY AND T. ADDIS. 1923. This Journal, lxv, 55.

THE CHEMICAL CONTROL OF BREATHING, AS SHOWN IN THE ACID BASE BALANCE OF THE BLOOD, UNDER PROGRESSIVE DECREASE OF OXYGEN[1]

YANDELL HENDERSON AND ELLEN M. RADLOFF[2]

From the Laboratory of Applied Physiology, Sheffield Scientific School, Yale University

Received for publication May 28, 1932

Oxygen deficiency induces an increase of breathing; but through what means or process this influence acts is still obscure. Is this influence, like that of carbon dioxide and that of the blood alkali exerted through the hydrogen ion concentration of the blood? Or does this factor dominate the others and exert a control of a different and more fundamental character?

The problem of the influence of oxygen deficiency is peculiarly difficult; this factor cannot be isolated experimentally. Its part in the chemical control of breathing cannot be studied apart from the influence of the other two factors, carbon dioxide and alkali, and their resultant hydrogen ion concentration. The effects of excess or deficiency of carbon dioxide are easily demonstrated apart from any other factor. The influence of increase or decrease of the alkali in use, or bicarbonate level of the blood, can also be shown independently. But the influence of oxygen deficiency upon respiration manifests itself essentially in the disturbances which it induces in the other factors. It can be brought into view and analyzed only by defining these disturbances.

ANOXEMIA AND ASPHYXIA; ALKALOSIS AND ACIDOSIS. In 1891 Araki (1) made an important and correct observation, which has nevertheless led to a vast deal of error and confusion. He found that in asphyxia, under carbon monoxide, there is a large production of lactic acid. This observation and similar observations under related conditions have been used ever since to explain the increased breathing under moderate decrease of oxygen. It is still a common, but quite erroneous, belief that a slight or moderate decrease of oxygen pressure leads to an increased production of lactic acid in the tissues, and that this acid escaping into the blood neutralizes a

[1] The experimental data upon which this paper is based are contained in a dissertation submitted by the junior author to the faculty of the Graduate School of Yale University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1930.

[2] Sterling Fellow.

part of its alkalinity, increases its hydrogen ion concentration and thus stimulates respiration. Moderate anoxemia has been confused with asphyxia, and has been conceived in terms of asphyxial acidosis.

In 1914 Barcroft (2) observed that under the lowered barometric and oxygen pressures on the Peak of Teneriffe, the alkali in the blood is diminished. But Ryffel (3) found no increase of lactic acid; and later confirmed this finding on men who spent four hours in a low pressure chamber where the oxygen was reduced to 11.8 per cent of an atmosphere. Marked over breathing occurred, but no increase of lactic acid. In similar experiments Haldane, Kellas and Kennaway (4) also found marked over breathing with no increase of lactic acid, but on the contrary, a decrease of acid and ammonia excretion in the urine: all indications of alkalosis. Henderson (5) independently reached the same conclusion on the basis of experiments on animals.

In a more recent study of the effects of the anoxemia of low barometric pressure Singer (6), using the pneumatic chamber in the laboratory of Professor Hess (7) at Zurich, confirms and extends these observations. He finds that at a barometric pressure of 360 mm., equivalent to 6,000 meters, cyanosis occurs in all persons. There is no decrease of basal metabolism, but a marked diuresis. There is hyperpnea, which Singer believes to be due to the influence of oxygen deficiency directly upon the respiratory center. In contrast to the effects of muscular exertion, the hyperpnea of anoxemia is not due to an acidotic alteration of the blood; on the contrary the hydrogen ion concentration, the titratable acidity and the ammonia of the urine are diminished. Singer finds that in normal men decreased pressure of oxygen—down to 75 mm.—induces no indications of an increased production of acid metabolites.

That oxygen deficiency might induce an acidosis confined to the nervous system is an idea to which Gesell (8) has devoted much experimental work. He argued that such an acidosis of the respiratory center might cause over breathing and thus induce an alkalosis of the blood. Gesell (9), however, in his more recent papers gives weight to the effects of oxygen deficiency upon the respiratory center through some process other than that of acidosis. His conception now appears to be somewhat like that formerly proposed by Loevenhart (10); it accepts oxygen deficiency as an influence independent of the hydrogen ion concentration. Respiration determines pH, instead of pH determining respiration. This conception is the exact opposite of that of Winterstein (11), that the pH is the hormone of respiration, yet, as we shall show later, both in a sense are true.

The opinion that the respiratory center and the nervous system generally might be subjected to an acidotic process, even when other parts of the body were subjected to alkalosis, is one that is on general grounds highly improbable. It is almost entirely lacking in support from valid evidence. The

only evidence for it comes from animals under experimental conditions of virtual asphyxia. In some of Gesell's (12) previous experiments the subjects, dogs, were drugged with heparin, morphine and urethane; their chests were opened and they were throughout the experiment under artificial respiration of uniform volume, but with periods of five to fifteen minutes of diminished oxygen supply. Observations under such conditions may throw light on some of the processes involved in asphyxia; but they are essentially misleading when applied to the problems of normal breathing in unoperated, unanesthetized men and animals.

Conclusive evidence against the theory of an acidosis in the respiratory center under a moderately decreased pressure of oxygen, as a possible cause of alkalosis in the blood, has recently been contributed by Myerson, Loman, Edwards and Dill (13). They have used a technique which enables them to obtain blood directly from the internal jugular vein in man. They find that even when the subjects were breathing air containing only 9 per cent of oxygen, 68 mm.,—the equivalent of an altitude of 22,000 feet—neither the venous blood from the brain nor from a limb contained any more than a normal amount of lactic acid or other acid metabolites.

The evidence from the literature thus demonstrates that mere anoxemia does not induce an increase of lactic acid formation or any other feature of acidosis. There probably is no such condition as anoxemic acidosis. The symptoms mistaken for those of acidosis—over breathing, lowering of the alveolar carbon dioxide and the gradual lowering also of the blood alkali—are not associated with an increase of the hydrogen ion concentration either in the blood or in the nervous system. The pH is raised, not lowered. The erroneous belief on this point still prevalent is based on the application of observations of increased lactic acid and lowered pH under conditions, not of mere anoxemia, but of asphyxia. It is only in asphyxia that an increased production of lactic acid occurs and the pH of the blood is lowered.

The influence of oxygen pressures below normal, but above 60 or 70 mm., is exerted upon the respiratory center, or upon its afferent end organs in the sinus caroticus, in a manner different from that of carbon dioxide and the blood alkali.

EXPERIMENTAL METHODS. In this investigation dogs were used throughout. Altogether twenty-three experiments were made, although all were not complete in all details. Two of the animals were narcotized with "amytal" (iso-amyl-ethyl-barbituric acid in half normal NaOH); but they were found so inert and unresponsive in respect to all physiological readjustments (see fig. 4) that this drug was not used further. Five of the animals received no general anesthetic; the others were given merely enough morphine to quiet them. But all of the animals were carefully protected from pain, anxiety, discomfort or any other condition that could excite

them to increase of respiration. Anoxemia is analgesic, and asphyxia is strongly anesthetic. In all cases novocain was used to anesthetize the skin, before the incisions were made to insert cannulae in the trachea and in the femoral artery for the withdrawal of blood samples. The volume of respiration was not recorded; but the general course of respiration can be inferred with considerable precision from the carbon dioxide content of the arterial blood.

In all cases as soon as the animal was tracheotomized the tracheal cannula was connected with inspiratory and expiratory valves. From one of these valves the animal expired through a cartridge of sodium hydroxide shells into a Douglas bag. Through the other valve the animal inspired again from the Douglas bag. There was thus a closed system containing such a volume of air that in the course of the experimental period the continual breathing of it reduced the oxygen content to a lethal percentage. In all of the experiments here reported, the expired carbon dioxide was absorbed so completely that the air reinspired from the Douglas bag contained not more than a few tenths of one per cent. The air in the bag was analyzed at intervals by means of a Henderson-Orsat analyzer for oxygen and carbon dioxide.

Except in a few cases where the dogs were quite small the blood samples were about 30 cc. They were drawn directly under oil into 3 cc. of an anticoagulant solution of 3 per cent potassium oxalate and 0.5 per cent sodium fluoride in 0.85 per cent saline. The samples were immediately chilled in a bath of ice water, to obviate the rapid acid change observed by Havard and Kerridge (14).

Determinations of the carbon dioxide content and capacity of the whole blood were made as quickly as possible after the samples were drawn on the constant volume apparatus by the manometric method of Van Slyke and Neill (15). Equilibrations were carried out in a water bath at 37° for twenty to thirty minutes at the carbon dioxide tensions specified in the figures. All the blood samples were fully oxygenated. The method used for estimating lactic acid was that of Friedemann, Cotonio and Shaffer (16); for sugar that of Folin and Wu (17); for inorganic phosphates that of Fiske and Subbarow (18). In determining the hydrogen ion concentration the Dale-Evans (19) modification of the colorimetric method of Levy, Rowntree and Marriott (20) was used. Dialysis tubes were made from a solution of "parlodion" in equal parts of alcohol and ether and dialysis was continued for twenty minutes at 37°C. The standard solution of pH 7.5 used for comparison was electrometrically standardized and was obtained from the La Motte Laboratories. In spite of the criticism of such colorimetric methods, especially by C. J. Johnston (21), the values obtained were considered satisfactory because of their consistency and because differences in pH rather than absolute values were of importance. Bayliss, Kerridge

and Verney (22) from comparison of results by the glass electrode and hydrogen electrode find the dialysis method is accurate to 0.02 pH.

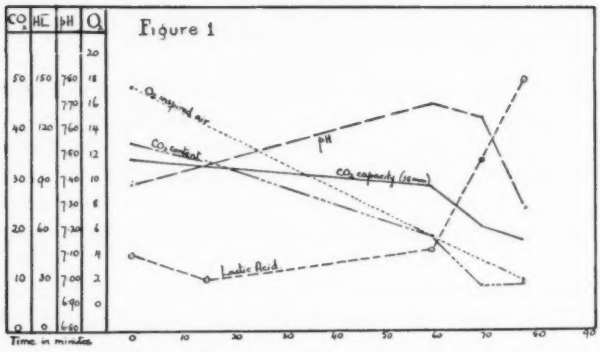
EXPERIMENTAL RESULTS. The principal results of these experiments are illustrated by figures 1, 2 and 3. Each shows the course of events throughout one experiment: the amount of oxygen in the inspired air, and the pH, carbon dioxide content, carbon dioxide capacity and lactic acid content of the blood.

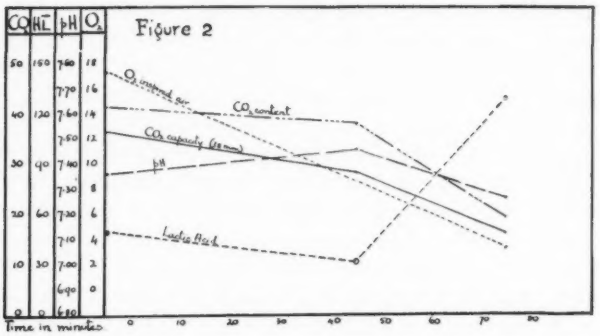
In all these experiments, as is clear from the figures, there are two distinct periods: a first period while the oxygen in the inspired air is falling, but has not yet reached a dangerous degree of depletion; and a second period after a dangerous depletion of oxygen has occurred. During this second period the depletion grows more and more harmful until death results from asphyxia. The critical level of oxygen pressure between these two periods is seen in the figures to be generally at, or a little below, 8 per cent of an atmosphere, 61 mm., of oxygen in the inspired air. This accords with the observations of Jervell (23) and of Mathison (24).

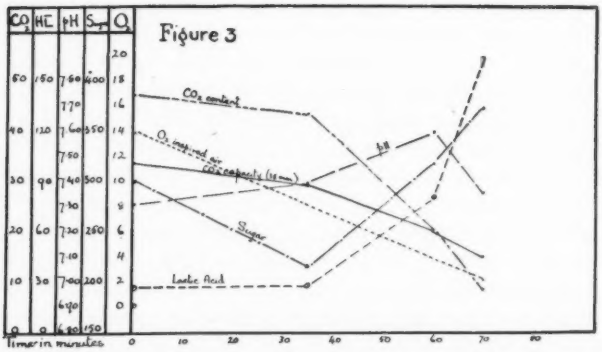
In the first period the significant fact is that as the oxygen falls, the pH does not fall. On the contrary, it rises. In other words, degrees of oxygen depletion above 8 per cent induce, not an acidosis, but an increasing alkalinity in the blood. Furthermore, throughout this first period, the lactic acid content of the blood shows no increase. On the contrary, it remains unchanged from the initial normal level.

The reason for the rise of pH is shown by the relation of the curves for the carbon dioxide content and carbon dioxide capacity; it is especially clear in figure 1. In this experiment the carbon dioxide content of the blood is seen to fall markedly during the first period, while the carbon dioxide capacity falls more slowly. The significance of these facts is that, when respiration begins to respond to a slight deprivation of oxygen, the pulmonary ventilation is increased and the alveolar carbon dioxide is decreased. Consequently the carbon dioxide content of the blood is decreased also and the pH of the blood rises: a rise evidently determined by the decrease in the ratio of $[H_2CO_3]:[BHCO_3]$. The rise of the pH would be much greater except for the fact that the carbon dioxide capacity of the blood, or blood alkali, undergoes a compensatory fall. But this lowering of the blood alkali is evidently not due to any such cause as an "anoxemic production of lactic acid," for throughout the first period no increase of lactic acid occurred in any of these experiments.

Turning now to the second period, that in which the inspired air contains less than 8 per cent of oxygen, we find very different phenomena and relations. The animals during this period are not merely making physiological responses to slight decrease of oxygen. On the contrary they are dying of asphyxia. The right hand ends of all the curves show the values for the various measurements at, or immediately before, death. During the







Figs. 1, 2 and 3. These figures show the course of events during progressive decrease of oxygen in three typical experiments. The two periods referred to in the text appear distinctly. In the first period the oxygen in the inspired air is above 8 per cent; in the second it is below 8 per cent. In the first over breathing is induced and the pH rises. From the curves for the CO_2 content and capacity, particularly in figures 1 and 3, it appears that this effect is due to the fact that decrease of the dissolved CO_2 is more rapid than the compensatory decrease of bicarbonates. The apparent discordance in figure 2 is probably due to slight errors of equilibration and analysis. During this first period the lactic acid undergoes no appreciable increase.

In the second period the pH, CO_2 content and capacity fall greatly and the lactic acid rises to a high figure. Death occurs at about 2 to 4 per cent of oxygen.

early part of this period the breathing is increased and the carbon dioxide content of the blood is correspondingly decreased. The carbon dioxide capacity, or blood alkali, falls rapidly to a figure far below normal, but not usually to a level that in itself would be fatal. Lactic acid appears in the blood in considerable, but not extremely toxic, amounts. The pH, which has risen in the first period, now falls considerably below normal although in no case to a level that by itself would necessarily be lethal.

In addition to these features there are two others, which are less evident, but even more important. In contrast to the increase of sensitivity noted in the period of anoxemia, the respiratory center during the period of asphyxia undergoes a progressive decrease of sensitivity. This development, which terminates in paralysis of breathing and death, is demonstrated by the lowered pH. Although the breathing may be greater than under normal conditions, its volume is nevertheless insufficient to keep the amount of carbonic acid in the blood down to equivalence with the decreased alkali. Hence the fall of pH. This fall shows that the respiratory center during asphyxia requires a progressively increasing stimulus to maintain its activity. In other words, the sensitivity of the center is steadily decreasing. If respiration were still equal to its duty, the overbreathing would be much greater than it is; and the relation of $[H_2CO_3]$: $[BHCO_3]$ in the blood, which determines the pH, would be kept normal in spite of the decrease of the alkali. This point will be more fully discussed in the next section.

The second important, but generally overlooked, development during the period of asphyxia is an exacerbation of the acapnia which is induced to a considerable extent even during the preceding period of anoxemia. In both periods carbon dioxide is thrown off faster than it is produced. This condition is easily overlooked and may have no great influence unless the supply of oxygen to the lungs is renewed in an effort to effect resuscitation. In that case, as we know from many other experiments in this laboratory, as soon as the depression of the respiratory center is partially relieved by a renewed supply of oxygen, acapnia makes itself felt in the lack of a stimulus adequate to induce breathing. Hence the life saving effect of inhalation of carbon dioxide diluted in air or oxygen after asphyxia. It supplies the requisite stimulus.

The sharp distinction here found between the first and second periods indicates that in the investigations of Gesell and others in which a considerable amount of lactic acid has appeared in the blood and the pH has fallen, the conditions were not those of a moderate decrease of oxygen within physiological limits, as in our first period; but were essentially the asphyxial and moribund conditions of our second period. It is probable, as Gesell believes, that acidosis develops in the center in asphyxia, as it does simultaneously in all active tissues under extreme deprivation of

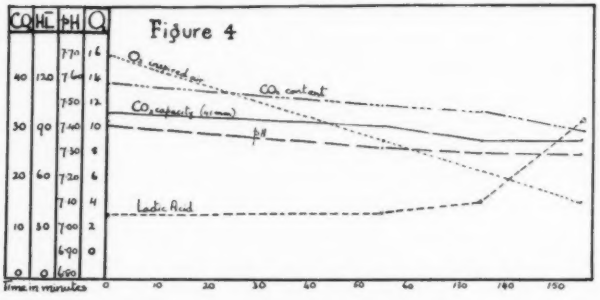


Fig. 4. This experiment, which was otherwise like the three preceding, shows the greatly decreased reactivity of an animal narcotized with a barbituric compound.

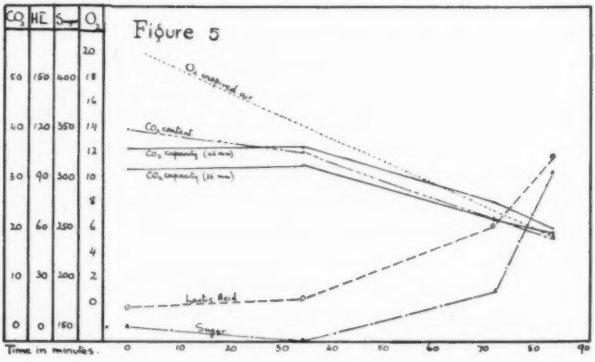


Fig. 5. In this experiment the conditions were like those in experiments 1, 2 and 3 except that both vagi had been cut.

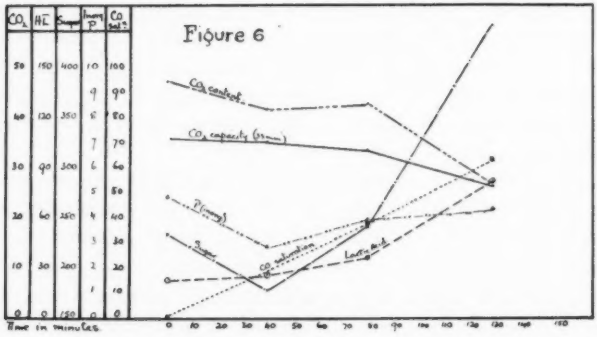


Fig. 6. In this experiment, instead of a progressive decrease of oxygen, the animal was subjected to a gradually increasing saturation of its blood with carbon monoxide.

oxygen. But our data show that this intracellular acidosis, instead of stimulating, is associated with a progressive depression of the respiratory and all other nerve centers. It terminates in respiratory failure and death.

In figure 4 are shown the data for an animal narcotized with amytal. The striking feature here is the almost complete absence of any response to decreased oxygen on the part of any of the functions observed; this is particularly the case in the first period of this experiment. Even in the second period the only distinct effects of acute asphyxia are an increase in lactic acid in the blood, a slight lowering of pH, and decrease both of the carbon dioxide content and capacity of the blood. In another investigation in this laboratory (Haggard and Greenberg (25)) a similar lack of change in the sugar content of the blood has been noted in dogs narcotized with amytal or other barbituric compounds. Evidently results in regard to respiration, blood gases and sugar metabolism obtained in experiments under this drug are to be regarded with doubt unless confirmed in other ways.

In figure 5 are shown the data from an animal in which both vagi were cut. The reactions of the functions here under study are seen to be not appreciably altered from those in animals with intact vagi. They are essentially the same as in the first three experiments above discussed.

In figure 6 are shown the data obtained from an animal in which deprivation of oxygen, leading to asphyxia and death, was induced by means of carbon monoxide. In this and other similar experiments the pure gas was prepared and was diluted down to a few tenths of 1 per cent with air. Unfortunately the pH was not determined. But it probably followed the same course as in the first three experiments above discussed. In respect to carbon dioxide content and capacity and lactic acid the effects were identical with those of the experiments in which the deprivation of oxygen was induced merely by rebreathing.

THE CRITICAL FACTORS IN ASPHYXIA. Ever since the conception of pH was introduced into chemistry, and from chemistry into biology and medical science, many physiologists and biochemists have made certain inferences from theory in regard to "acidosis," which account satisfactorily for some facts, but which largely ignore some others, no less important but of contrary implication. It is true that in asphyxia and related conditions lactic acid appears in the blood, the blood alkali is decreased, and the pH is lowered. It has been inferred therefore that in asphyxial acidosis and related conditions the body is intoxicated by acid. The part played by respiration has been largely misunderstood or ignored.

In many forms of acidosis the increase of lactic acid, or other acid elements, and the decrease of the blood alkali are really quite insufficient to cause such a perversion of pH as occurs. They are indeed insufficient to force any perversion whatever, if there were not some other factor also

acting. This statement is particularly true, as our data show, in asphyxial acidosis. The abnormal factor is depression of respiration: a depression too slight to be observed in any effect except a pH below normal.

Respiration has a sufficient "factor of safety" to compensate for very wide variations of blood alkali, and thus to prevent a lowering of pH. If in any way the blood alkali is decreased to a half, or a third, or a quarter of its normal value, it is only necessary for the dilution ratio of breathing—the volume of air breathed per unit mass of carbon dioxide exhaled—to be increased to double or threefold or fourfold: the pH would thus be held at its previous normal value. Respiration is capable of a much greater variation of activity than any such adjustment ever requires, short of such extreme conditions as in the Kussmaul breathing of diabetic coma (26). It follows therefore that, if respiration does not effect a complete compensation of an abnormally lowered pH, the cause lies in a depression of the sensitivity of the respiratory center. In other words, respiration is not maintaining a sufficient ventilation of carbon dioxide out of the blood in the lungs. The sensitivity of the respiratory center is depressed.

This conception has very great advantages for the analysis of many respiratory problems. It reduces the otherwise vague idea of the chemical control of breathing to two clear-cut factors: 1, the pH of the blood as the specific stimulus to the respiratory center, as postulated by Winterstein (11), and 2, the sensitivity of the respiratory center to this stimulus. Neither of these factors is adequate alone to account for the control of breathing. Both are necessary. Something of this sort is indeed now recognized; for instance, Peters and Van Slyke (27) say that the pH of the blood "shows whether the respiratory apparatus is operating in the normal manner to prevent any considerable change of the $[BHCO_3]:[H_2CO_3]$ ratio in the blood." But these authors consider that "the normal behavior of respiration does not necessarily entail the maintenance of a normal pH. When the bicarbonate of the plasma is reduced . . . pulmonary ventilation does not become accelerated enough to compensate entirely . . . so as to maintain pH unchanged."

This statement is well founded both experimentally and clinically. We suggest that it be amended only by substituting the words "basal pH" for "normal pH." Respiration maintains a basal pH only under basal conditions. Muscular exercise, anoxemia, mental excitement, asphyxia, ether anesthesia, morphine narcosis, and many other conditions, both normal and abnormal, alter the sensitivity of the center. Any increase or decrease of sensitivity to hydrogen ions induces a corresponding change in the pH of the blood. The pH of the blood is the expression and index of the sensitivity of the respiratory center.

According to this conception, the well established data concerning the effects of vigorous muscular exercise upon the ratio $[H_2CO_3]:[BHCO_3]$ in

the blood plasma should be interpreted as showing that the sensitivity of the center is increased during the exercise and decreased in the subsequent period of rest. The pH after exercise, as Douglas and Havard (28) have recently pointed out, is distinctly below the basal value; yet the subject breathes quietly. If the sensitivity of the respiratory center were constant at its basal value the high concentration of hydrogen ions in the blood during rest after exercise would induce vigorous hyperpnea.

With this conception in mind and largely on the basis of evidence to be referred to in the next section, we are led to conclude that the really critical factors in asphyxia are not the increase of lactic acid, nor the low pH of the blood, nor any other feature of acidosis. None of these conditions, nor all of them together, are sufficiently intense to account for the depression of respiration and other functions, and finally death, in asphyxia. The really critical factors are the depression of the sensitivity of the respiratory center and the acapnia, which has been induced by over breathing, and which leaves the depressed respiratory center with an inadequate stimulus. In other words, respiration does not fail, because the pH of the blood is so low that the system is poisoned by acidosis. On the contrary, it fails because the pH, although low, is not low enough to overcome the depression and to stimulate the center.

There is direct experimental evidence for this conception. Haldane (29) observed that animals, which have collapsed from carbon monoxide asphyxia, are partially revived when carbon dioxide is added to the atmosphere in which the asphyxia has been induced. Haggard (30) independently has shown that animals in an asphyxiant atmosphere of carbon monoxide reach a greater degree of saturation with that gas, before death is induced, when a considerable amount of carbon dioxide is also present in the atmosphere, than without it.

That asphyxial acidosis is a very different condition from the acidosis induced by intravenous injection of hydrochloric acid is shown by observations of Haggard and Henderson (31). They found that in the acidosis induced by intravenous injection of hydrochloric acid the inhalation of carbon dioxide is quickly fatal—exactly as the acidosis theory requires; but that two to three times as much lactic acid as hydrochloric acid, molecule for molecule, is required to induce acid poisoning. The body itself never produces such an amount.

Resuscitation from asphyxia. There are strong clinical, as well as experimental, reasons for postulating variability in the sensitivity of the respiratory center, and for regarding depression of this sensitivity as one of the two critical factors in asphyxia. These reasons are afforded by experience in this laboratory in connection with the problem of resuscitation. For more than twenty-five years investigations in this laboratory have been devoted to the development of the method of resuscitation from post-

operative depression and other forms of asphyxia and acapnia by means of inhalation of carbon dioxide (32), diluted in air or in oxygen. In these investigations the fundamental ideas have been 1, to counteract the acapnia which, except in suffocation and drowning, always develops in asphyxia, and 2, to overcome the depression of the respiratory center by a supernormal stimulus. The inhaled carbon dioxide stimulates the asphyxiated center into activity; the oxygen, which this activity supplies, gradually restores a normal sensitivity in the center; the inhaled carbon dioxide then counteracts the acapnia which would otherwise render even a normally sensitive center apneic.

This idea has been applied successfully to resuscitation from carbon monoxide asphyxia (33), from post anesthetic depression (34), from asphyxia in the newborn (35), and from many related conditions. Depression of the respiratory center is the principal feature of drowning. Depression and the acapnia, which results from displacement of carbon dioxide from the bicarbonates of the blood by lactic acid in asphyxial acidosis, are the critical features in the asphyxia of the newborn. In these and other forms of asphyxia inhalation of carbon dioxide, diluted in air or oxygen, is now coming to be recognized as the specific means of resuscitation. It stimulates the respiratory center and counteracts acapnia. Experience for a decade past has proved its effectiveness for carbon monoxide asphyxia, and for post anesthetic and post operative depression (36).

In its various applications this method of resuscitation is now annually saving hundreds of lives. Yet if the conception that acidosis is the critical factor in asphyxia were correct, inhalation of carbon dioxide should certainly harm, not help. It should kill, not resuscitate. This method of resuscitation has in fact been repeatedly opposed on this theoretical ground. If the objection were based on facts, instead of an erroneous inference from the theory of the acid base equilibrium of the blood, then indeed it would be true as a recent, and quite logical, supporter of (37) the acidosis conception has expressed it, that "the use of carbon dioxide as a resuscitating agent in asphyxia neonatorum is not only superfluous, but may even be harmful, in that it tends to aggravate an already existing acidosis." Furthermore the alleged superfluousness and harmfulness of inhalation of carbon dioxide would apply equally to all other forms of asphyxia.

PRINCIPAL CONCLUSIONS

When the oxygen content of the inspired air is gradually reduced without accumulation of carbon dioxide, the effects develop in two distinct periods. One of these periods is that while the percentage of oxygen in the inspired air is above 8 per cent. The other period occurs after the oxygen has fallen below 8 per cent.

The first period involves merely anoxemia of tolerable degrees. Respira-

tion increases; the carbon dioxide content of the blood is decreased; and the pH rises. In compensation the blood alkali is automatically, but more slowly, decreased. In these reactions lactic acid plays no part. On the contrary, the amount of lactic acid in the blood remains at a normal level. The conditions induce, not acidosis, but alkalosis.

The second period is that of asphyxia and ends in death. The carbon dioxide content and capacity of the blood fall further; the lactic acid content is considerably increased; and the pH falls, with the development of acidosis.

The increase of breathing during the first period is clearly not due to formation of lactic acid and increase of the hydrogen ion concentration in the blood; nor is there any valid evidence for an acidosis localized in the respiratory center. The common, but erroneous, belief that moderate degrees of oxygen deficiency stimulate respiration through acidosis comes from observations on the acidosis which develops only under asphyxia. The belief is not valid even for this period; for under asphyxial acidosis the respiratory center is not stimulated but depressed. Moderate oxygen deficiency does not exert its influence upon breathing through acidosis and an increase of hydrogen ion concentration.

The conception of the chemical control of breathing here presented is as follows: The specific stimulus to the respiratory center is not carbon dioxide, but the hydrogen ion concentration of the blood plasma. The concentration of hydrogen ions is not an independent factor, but is the resultant of two factors, each of which controls a particular feature of respiration. The blood alkali determines the dilution ratio of breathing: that is, the volume of air breathed per unit mass of carbon dioxide exhaled. The carbon dioxide production of the body determines the volume of air breathed at the dilution ratio set by the alkali in use. But these controls operate to afford a basal pH only so long as the oxygen supply is ample and no other disturbing influences occur to alter the sensitivity of the respiratory center from the value that it has under basal conditions. The sensitivity sets the pH to which the respiratory center will respond and which it will therefore maintain.

Oxygen deficiency, through some non-acidotic process, increases the sensitivity of the respiratory center to its specific stimulus: the hydrogen ion concentration of the blood plasma. The center then maintains an increased volume of breathing which raises the pH of the blood. After muscular exercise to a slight degree and in asphyxia to an intense degree the sensitivity of the respiratory center is decreased. In consequence the volume of breathing is decreased below the amount, and the pressure of carbon dioxide and concentration of H_2CO_3 rise above the amount, that would correspond to the alkali in use in the blood. The pH of the blood is thus lowered. A restoration of normal sensitivity in the center and a

volume of breathing increased sufficiently to compensate for the decreased blood alkali would immediately restore a basal pH. Except under the most extreme variations of the blood alkali in disease, acidosis, in the sense of low pH, is always primarily evidence of depression of the respiratory center.

Chemically the pH of the blood expresses the balance of acids and bases. Emphasis upon this relation has tended to conceal another relation which is equally important. Physiologically the pH of the blood is an index of the sensitivity of the respiratory center.

BIBLIOGRAPHY

(1) Araki, T. 1891. Zeitschr. f. physiol. Chem., xv, 335; 1894, xix, 422.
(2) Barcroft, J. 1914. Respiratory function of the blood. Cambridge Univ. Press. 1925. Respiratory function of the blood. Part I. Lessons from high altitudes.
(3) Ryffel, J. H. 1910. Journ. Physiol., Proc. Physiol. Soc., p. xxix.
(4) Haldane, J. S., A. M. Kellas and E. L. Kennaway. 1919. Journ. Physiol., liii, 181.
(5) Henderson, Y. 1919. Science N. S., xlix, 431. 1920. Journ. Biol. Chem., xliii, 29. 1932. Nature, cxxix, 649.
(6) Singer, W. 1929. Zeitschr. f. gesammt. exper. Med., lxvi, 45; 1931, lxxviii, 712.
(7) Hess, W. R. 1931. Die Regulierung der Atmung. Leipzig.
(8) Gesell, R. 1925. Physiol. Rev., v, 551.
(9) Gesell, R., H. Krueger, H. Nicholson, C. Brassfield and M. Pelecovich. 1932. This Journal, c, 202, 227.
Haldi, J. 1932. Ibid., xcix, 702.
(10) Loevenhart, A. S. 1915. Arch. Int. Med., xv, 1059.
(11) Winterstein, H. 1911. Pflüger's Arch., cxxxviii, 167. Winterstein, H. and Gollwitzer-Meier. 1928. Pflüger's Arch., ccxix, 202.
(12) Gesell, R., H. Krueger, G. Gorham and T. Bernthal. 1930. This Journal, xciv, 300, 339, 365, 387, 402.
(13) Myerson, H., J. Loman, H. T. Edwards and D. B. Dill. 1931. This Journal, xcvii, 373.
(14) Havard, R. E. and P. T. Kerridge. 1929. Biochem. Journ., xxiii, 600.
(15) Van Slyke, D. D. and J. M. Neill. 1924. Journ. Biol. Chem., lxi, 523.
(16) Friedemann, T. E., M. Cotonio and P. A. Shaffer. 1927. Journ. Biol. Chem., lxxiii, 335.
(17) Folin, O. and H. Wu. 1920. Journ. Biol. Chem., xli, 367.
(18) Fisk, C. H. and Y. Subbarow. 1925. Journ. Biol. Chem., lxvi, 395.
(19) Dale, H. H. and C. L. Evans. 1920. Journ. Physiol., liv, 167. Ibid., 1922, lvi, 125.
(20) Levy, R. L., L. G. Rowntree and W. McK. Marriott. 1915. Arch. Int. Med., xvi, 389.
(21) Johnston, C. J. 1928. Journ. Biol. Chem., lxxix, 297.
(22) Bayliss, L. E., P. T. Kerridge and R. C. Verney. 1926. Journ. Physiol., lxi, 448.
(23) Jervell, O. 1928. Acta Med. Scand., Suppl., xxiv-xxvi, 65.
(24) Mathison, G. C. 1910. Heart, ii, 54. 1911. Journ. Physiol., xlii, 283.

(25) HAGGARD, H. W. AND L. A. GREENBERG. Personal communication.
(26) HENDERSON, Y. 1932. Practioner's Library of Medicine and Surgery, New York. I. 751, Chapter xi. The principles controlling respiration in health and disease.
(27) PETERS, J. P. AND D. D. VAN SLYKE. 1932. Quantitative clinical chemistry, i, 939. Baltimore.
(28) DOUGLAS, C. G. AND R. E. HAVARD. 1932. Journ. Physiol., lxxiv, 471.
(29) HALDANE, J. S. 1922. Respiration. Yale University Press, 363.
(30) HAGGARD, H. W. 1921. This Journal, lvi, 390.
(31) HAGGARD, H. W. AND Y. HENDERSON. 1920. Journ. Biol. Chem., xliii, 3.
(32) HENDERSON, Y. 1906. Brit. Med. Journ., ii, 1812. 1919. Bull. Johns Hopkins Hosp., xxi, 235. 1908–1919. This Journal, xxi, 126; xxiii, 345; xxiv, 66; xxv, 310; 385; xxvi, 260; xxvii, 152; xlvi, 533.
(33) HENDERSON, Y. AND H. W. HAGGARD. 1922. Journ. Amer. Med. Assoc., lxxix, 1137.
HENDERSON, Y. 1924. Ibid., lxxxiii, 758; 1930, xciv, 179.
(34) HENDERSON, Y., H. W. HAGGARD AND R. C. COBURN. 1920. Journ. Amer. Med. Assoc., lxxiv, 783.
(35) HENDERSON, Y. 1928. Journ. Amer. Med. Assoc., xc, 583; 1931, xcvi, 495.
McILROY. 1927. Lancet, ii, 373.
WIENER, R. 1931. Arch. Kinderheilkunde, xcv, 65.
(36) HENDERSON, Y. 1931. Brit. Med. Journ., 687.
(37) EASTMAN, N. J. 1932. Bull. Johns Hopkins Hosp., l, 39.

STUDIES ON SUPRARENAL INSUFFICIENCY

XI. The Growth of Transplanted Cortical Tissue in the Rat

LELAND C. WYMAN and CAROLINE TUM SUDEN

From the Physiological Laboratory of Boston University School of Medicine and the Evans Memorial, Mass. Memorial Hospitals

Received for publication May 31, 1932

It has been conclusively demonstrated by Jaffe and Plavska (1926) and Jaffe (1927a) that free autoplastic transplants of suprarenal cortex will grow and indefinitely maintain the health of suprarenalectomized rats and guinea pigs. Successful takes in rats had been reported by Poll as early as 1898, and some evidence that cortical transplants function in the rabbit had been presented by Busch, Leonard and Wright in 1908. Discussions of suprarenal transplantation may be found in the reviews by Jaffe (1927b) and Britton (1930). The technique for rats devised by Jaffe and Plavska has been used successfully by numerous investigators ever since 1926.

In spite of these facts the success of the earlier workers seems to be ignored by some recent writers, and such statements as the following occasionally appear in the literature: "Attempts to transplant the adrenal cortex have been made repeatedly, but on the whole the few 'takes' reported are open to criticism" (Johnson and Johnson, 1931), and "Auto-transplants of practically every tissue in the body have been attempted with much success. Auto-transplantation of the adrenal, however, has not met with such success" (Oldberg, 1929). Recent texts also disregard the work with rats and guinea pigs, or quote less conclusive experiments. *Human Physiology* by Winton and Bayliss (1931) contains this statement: "The complete proof of the specific secretory function of the gland would depend on the cure of adrenalectomized animals by transplanting cortical tissue from a normal animal; unfortunately, however, such grafting has been unsuccessful, the tissue degenerating in a short time."

During the past five years autoplastic transplantation of the suprarenal cortex in rats by the technique of Jaffe and Plavska has been used successfully in this laboratory in a series of studies on suprarenal insufficiency. Both glands are removed, cut in half and the four parts transplanted into pockets in the abdominal muscle. From one to four of these transplants regenerate as masses of cortical tissue. It was noticed at autopsy that when one only of the transplants had regenerated it was almost invariably

larger than those seen when two or more had regenerated. Moreover, small size of regenerated transplants was often associated with the presence of accessory masses of cortical tissue within the abdominal cavity (the term "accessory" is used in this paper to refer to any gross mass of cortical tissue found within the abdominal cavity, irrespective of whether it may have regenerated from a fragment of the gland left at operation or from a true accessory cortical rest). This led us to suspect a physiological limitation with respect to the amount of cortical tissue which can regenerate from transplants or cortical rests. Accordingly a series of cortical transplantations made in various ways was studied and the results are reported below.

Autotransplantation. In order to get a figure which would express the relative amount of cortical tissue regenerating in transplanted or accessory masses three diameters of each mass found at autopsy were measured in millimeters; these were multiplied and the sum of these products for each animal was taken as an expression of the "volume" of regenerated cortical tissue present. It is realized that such a method is subject to considerable error, but the results showed that these figures were comparable, and that they roughly expressed what could plainly be seen at autopsy with regard to the relation of size of cortical masses to the number present. Data from transplantations done during the past five years were gathered in this way. In the recent experiments the cortical masses were also weighed in milligrams, and the weights were found to correspond with the figures obtained from the measurements.

Suprarenalectomy and transplantation were done on young rats from 45 to 90 days of age in 100 cases, and from 90 to 120 days of age in 43 cases. No correlation was found between the age at which transplantation was done and the growth of transplanted or accessory cortical tissue. Autopsies were performed during the third month after operation in 36 cases, during the fourth month in 57 cases, during the fifth month in 34 cases, and during the seventh, eighth and tenth months in the remaining 16 cases. Although the ranges for the amount of regenerated cortical tissue for those rats autopsied during the third and fourth months were the same, a greater percentage of the rats fell in the lower half of the range during the third month. The difference was not great enough to affect the curves for all the data. It is safe to say that regenerated cortical tissue is fully established by the fourth month, and from this time on there is little if any further growth.

A definite sex difference in the capacity to regenerate cortical tissue was found. Females regenerated over twice as much as males. The average "volume" for 69 female transplants was 47.8, and for 72 male transplants it was 20.5. The average weight for 31 females was 28 mgm., and for 29 males it was 11.6 mgm. This sex difference is shown in figures

1 and 2. It is well known that the suprarenal glands of female rats and mice are larger than those of males. Anyone who is familiar with this difference can usually tell the sex of a rat by looking at the suprarenals alone.

From one to four masses of cortical tissue may be present in a transplanted animal. These may be successful transplants, accessory masses

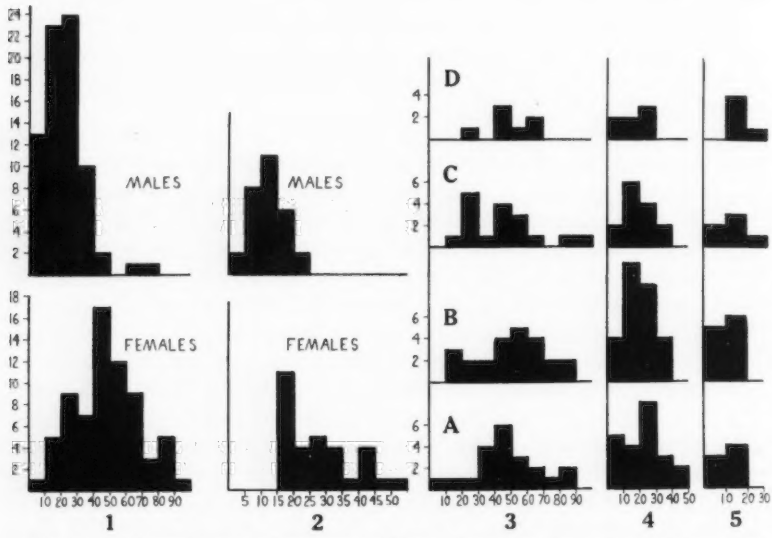


Fig. 1. "Volumes" (sum of products of three diameters; see text) of regenerated cortical tissue in 74 male and 69 female transplants. Abscissae represent "volumes." In this and in subsequent figures ordinates represent number of cases.

Fig. 2. Weights of regenerated cortical tissue in 29 male and 31 female transplants. Abscissae, weights in milligrams.

Fig. 3. "Volumes" of regenerated cortical masses in 69 female transplants. Abscissae, "volumes." In this and in figures 4 and 5: A, rats with one regenerated mass of cortical tissue; B, rats with two masses; C, rats with three masses; D, rats with four masses.

Fig. 4. "Volumes" of regenerated cortical masses in 71 male transplants. Abscissae, "volumes."

Fig. 5. Weights of regenerated cortical masses in 29 male transplants. Abscissae weights in milligrams.

and transplants, or accessory masses alone, the transplants having disappeared. When one mass only is present it is large, sometimes fully twice as large as a normal suprarenal gland. When two or more masses are present they are correspondingly smaller. Often one or two very small transplants are accompanied by one or two larger accessory masses. On

account of the sex difference described above, figures for the two sexes have to be considered separately. Although there is considerable individual variation, figures 3, 4 and 5 show that in general the total amount of cortical tissue regenerating appears to be fairly constant, irrespective of the number of masses present. The data were obtained from 109 autoplastic transplants made in the usual way, of which 23 had regenerated one transplant, 25 two transplants, 14 three transplants, and 11 four transplants, while 36 had one, two or no transplants together with one or two accessory masses. In addition 12 rats were deliberately transplanted with a single half gland and 22 received two transplants, each being half of a single gland. Of these latter 6 regenerated one and 16 regenerated both transplants.

Apparently in the rat there is a limiting factor, differing in the sexes, which regulates the amount of suprarenal cortical tissue which can regenerate from transplants, from fragments left at suprarenalectomy, or from cortical rests. This should be taken into consideration in studying the phenomenon of compensatory hypertrophy of cortical tissue. It may also have some bearing on the theory involved in experiments on the dosage of cortical extracts, inasmuch as it is probably a physiological or functional limitation. Obviously the transplantation method cannot be used to induce hyperfunction of the suprarenal cortex.

Autotransplantation and single suprarenalectomy. In three male rats, 48 days old, one suprarenal was removed and transplanted in two pieces into the abdominal muscles, and the other was left intact. At autopsy, 113 to 127 days later, no transplants were found. In five male and four female rats, 76 days old, one suprarenal was similarly transplanted, and a portion of the other was resected, leaving from one-quarter to one-half of the gland. At autopsy, 130 to 134 days later, no transplants were found and the portion of the suprarenal left in situ had regenerated into a mass about the size of a normal gland. Evidently the presence of one suprarenal, or even a good sized fragment of cortical tissue, completely inhibits the growth of autoplastic transplants of cortical tissue. This is probably simply another expression of the physiological limitation with respect to growth of cortical tissue, and it may account for the failure of certain previous investigators to get successful "takes" when one gland was transplanted and the other was left to maintain the animal during the process of regeneration of the transplants.

Homotransplantation. Four male rats were suprarenalectomized at 38 and 45 days of age and each was transplanted with the suprarenal glands (in four pieces) from a litter mate. In all four cases the homotransplants regenerated and apparently functioned. At autopsy, 81 to 126 days after operation, it was found that one, three and four (two cases) of the four pieces transplanted had regenerated respectively.

In order to see if both autoplastic and homoplastic transplants would grow equally well in the same animal, one in the presence of the other, or if there would be a "preference" when both types of tissue were transplanted, ten male rats, 48 to 62 days of age, were suprarenalectomized and each was transplanted with one of its own suprarenal glands (in two pieces) on the right side of the abdomen and with a gland from a litter mate on the left side. At autopsy, from 87 to 121 days after operation, it was found that in six cases one or two autoplastic transplants only had regenerated, and in four cases one or two of each type of transplant had regenerated. In these latter cases there was no apparent difference between the autoplastic and the homoplastic transplants. Evidently the presence of an autoplastic transplant does not inhibit the growth of a homoplastic transplant, and there is apparently no great "preference" when both types of tissue are offered.

Three female and four male rats, from 45 to 50 days of age, were transplanted in the abdominal muscles with four (4 cases) or 6 (3 cases) half suprarenal glands from litter mates, leaving their own glands intact. At autopsy, from 113 to 127 days after operation, no signs of these transplants were found. The rats' own glands were apparently normal. The presence of intact suprarenal glands inhibits the growth of homoplastic transplants. This eliminates the possibility of studying excessive cortical function by means of "super-transplantation."

Jaffe (1927b) has reported that of fifteen rats examined one month after homotransplantation four had positive transplants.

Heterotransplantation. Whole suprarenal glands cut into two pieces from young and adult female mice were transplanted into the abdominal muscles of seven rats, 100 days old, immediately following suprarenalectomy of the host animals. Fragments of suprarenal cortex of various sizes from a rabbit and from a guinea pig were similarly transplanted into suprarenalectomized rats (seven cases for each species). In no case was there any evidence of growth or function of these heterotransplants, and at autopsy from 23 to 217 days after operation no signs of the transplanted tissues were found, the sites of insertion being perfectly clean.

Body growth. A study of the growth curves of 58 cases showed that the growth rate of suprarenalectomized rats having autoplastic or homoplastic transplants, or accessory cortical tissue was the same as that of normal rats of the same sex and age period. It was also seen that after the 80th or 90th day of life the growth rate of females decreased decidedly more than that of males. In 6 cases of suprarenalectomized males having no gross cortical tissue, during a period from the 90th to the 180th day of life, the growth rate fell to the normal female level. Except for these 6 cases, the data for the group as a whole agree closely with those given by Donaldson (1915).

SUMMARY

1. Suprarenalectomized female rats having autoplastic transplants of cortical tissue, "accessory" cortical tissue, or both, regenerate more cortical tissue than do male rats.

2. The total amount of cortical tissue regenerating in such animals is fairly constant for each sex, irrespective of the number of masses present.

3. Homoplastic transplants of cortical tissue in rats are successful, and will grow in the presence of autoplastic transplants made at the same time.

4. Heteroplastic transplants of suprarenal cortical tissue in rats are unsuccessful.

5. Neither autoplastic nor homoplastic transplants of cortical tissue will grow in the presence of both, one, or even a good sized fragment of one of the animal's own suprarenal glands.

6. It is suggested that there is a physiological or functional factor limiting the growth of suprarenal cortical tissue in transplants or accessories. The transplantation method cannot be used to induce hyperfunction of the suprarenal cortex.

BIBLIOGRAPHY

BRITTON, S. W. 1930. Physiol. Reviews, x, 617.

BUSCH, F. C., T. M. LEONARD AND T. WRIGHT. 1908. Journ. Amer. Med. Assoc., li, 640.

DONALDSON, H. H. 1915. The rat. Memoir no. 6 of The Wistar Institute.

JAFFE, H. L. 1927a. Journ. Exper. Med., xlv, 587.

1927b. Arch. Pathol. and Lab. Med., iii, 414.

JAFFE, H. L. AND A. PLAVSKA. 1926. Proc. Soc. Exper. Biol. and Med., xxiii, 528.

JOHNSON, A. AND V. JOHNSON. 1931. This Journal, xcvii, 392.

OLDBERG, E. 1929. This Journal, xci, 275.

POLL, H. 1898. Zentralbl. f. Physiol., xii, 321.

WINTON, F. R. AND L. E. BAYLISS. 1931. Human physiology. Philadelphia.

CEREBROSPINAL ELASTICITY IN THE CAT AND MACAQUE

LEWIS H. WEED AND LOUIS B. FLEXNER

From the Department of Anatomy, Johns Hopkins University

Received for publication June 1, 1932

The possibility of applying with profit, to the dural sac and its contents, the customary physical formula for the determination of the coefficient of elasticity has been presented in former papers (1), (4). This formula is given as the quotient obtained by dividing the stress by the strain—i.e., the difference in pressure divided by the quotient of the difference in volume by the original volume, or $E = dP \Big/ \frac{dV}{V} = V \frac{dP}{dV}$. The E in this formula may be taken as a general coefficient of elasticity of the system as a whole and should not be confused with Young's Modulus (linear stretch), to which it can be simply related only in spherical or cylindrical systems.

The application of this general formula to the elasticity of the dural sac and its contents came through analysis of data yielded by experiments dealing with the abrupt tilting of dogs from the horizontal to the vertical (head-down, tail-down) positions. In one phase of this study, the pressure of the cerebrospinal fluid was determined by the bubble manometer (permitting measurement without dislocation of fluid) and by open-end manometers of different bore. It was found that as the bore of the manometer was increased the pressure-changes on tilting became less, though the volume of fluid dislocated into or from the manometer became greater. These differences in pressure-change (dP) and in volume-change (dV) were found to have a definite relationship to each other; the fraction dV/dP was ascertained to be of fairly constant value in any one dog or in those of the same size and age (4). With determination of the fraction dV/dP in any one animal and subsequent measurement of the intradural contents (cranial and spinal, for the total volume V), substitutions could be made in the formula $E = V \frac{dP}{dV}$ and the coefficient of elasticity established.

By this means, the coefficients of elasticity of a series of twenty dogs of different sizes and ages were computed and the results reported (1). The group of immature or juvenile animals was found to have a coefficient of elasticity which averaged 4.58×10^5 dynes per cm.^{2}; the young adults, 4.22×10^5 dynes per cm.^{2}; the adults, 4.03×10^5 dynes per cm.^{2}; and the

obviously old animals, 3.81×10^5 dynes per cm.^{2} In spite of the many difficulties of accurate classification of dogs according to age, the four groups showed great constancy in the value of the coefficient of elasticity and only one animal out of twenty yielded values not in keeping with the others of the same age-group.

The present report deals with the continuation of the study upon two other common laboratory mammals, the cat and the macaque. These animals were selected as the cat serves as a control to the findings on the dog, and as the macaque is essentially an animal of erect posture. The experiments were all performed as in the previous work, under ether anesthesia and with the tilting carried out so that the pressure of the cerebrospinal fluid could be recorded with open-end manometers of different bore. At the conclusion of the observations the animals were killed and the total intradural contents measured.

EXPERIMENTAL FINDINGS. With the determination of the pressure-changes on tilting to the vertical head-down and tail-down positions, and with the volume-changes derived from the calibrated manometers, it was possible to compute the fraction dV/dP for each manometer, after comparison with the volume and pressure-alterations recorded for the 1 mm. manometer. Four of these calculations were made from the data obtained in the head-down tiltings (manometers of 4, 6, 8 and 10 mm.) and similarly four calculations were made in the tail-down tiltings. Each of these series of four values was averaged, and finally a general average of all eight readings taken. Transient changes in elasticity (largely due to variations in the light surgical anesthesia considered most desirable) led to slight variations in the individual values of dV/dP for the different manometers but on the whole the results showed the same degree of constancy as was reported for the dog. As the coefficients of elasticity are somewhat different for the cat and the macaque, the findings will be presented separately for the two animals.

Cats. Table 1 gives data regarding the coefficient of elasticity of the dural sac and its contents in the series of 15 cats used. The fraction dV/dP ranged from 0.064 in an immature animal of 2220 grams to 0.117 in an adult of 2370 grams, while the coefficient of elasticity was found to vary between 3.86×10^5 dynes per cm.^{2} in the same juvenile to 3.26×10^5 dynes per cm.^{2} in an adult. The table shows a fair degree of constancy in the coefficient of elasticity within the various age-groups, the youngest group averaging 3.81×10^5 dynes per cm.^{2}; the young adult group, 3.56×10^5 dynes per cm.^{2}; the wholly adult group, 3.38×10^5 dynes per cm.^{2} No obvious relationship between spinal length, body weight, intradural volume, and the coefficient of elasticity is apparent in the data comprising table 1.

In the cats, as in the dogs, the values of the fraction dV/dP were roughly of the same magnitude whether derived from data furnished by the head-

down tiltings or by tail-down tiltings. This similarity in the two values of the fraction was shown in many of the fifteen cats included in table 1, as for instance in cat C 8, where the head-down calculation of dV/dP was 0.100 and the tail-down, 0.098. Again, in cat C 57, the head-down fraction was 0.075 and the tail-down, 0.073; and in cat C 60, the head-down derivation yielded 0.077 and the tail-down 0.071. These three examples show a slightly larger fraction for the head-down tiltings than for the tail-down; for the whole series this generalization held as the average value of dV/dP obtained from data of head-down tiltings was 0.088 while that from the tail-down tiltings was 0.084. Occasional animals (as cat C 39) gave calculation of the fraction for the tail-down tiltings in excess of those of the

TABLE 1

Coefficient of elasticity of the dural sac and its contents in cats

EXPERIMENT NUMBER	WEIGHT	SPINAL LENGTH	INTRADURAL VOLUME	$\frac{dV}{dP}$	COEFFICIENT OF ELASTICITY	AGE-GROUP
	grams	*mm.*	*cc.*		*dynes per cm.²*	
C 61	2,220	333	25.1	0.064	3.86×10^5	Immature
C 60	2,120	322	28.2	0.074	3.76×10^5	Immature
C 57	2,640	342	28.6	0.074	3.81×10^5	Immature
C 53	2,170	327	29.0	0.079	3.62×10^5	Young adult
C 51	2,480	358	28.7	0.082	3.45×10^5	Young adult
C 46	2,090	324	33.6	0.094	3.55×10^5	Young adult
C 9	2,220	325	35.2	0.095	3.65×10^5	Young adult
C 1	2,755	360	36.0	0.098	3.62×10^5	Young adult
C 8	3,420	340	36.4	0.099	3.62×10^5	Young adult
C 39	2,650	340	38.5	0.110	3.43×10^5	Young adult
C 42	2,380	345	28.4	0.085	3.29×10^5	Adult
C 50	3,060	382	30.8	0.086	3.53×10^5	Adult
C 54	3,070	352	31.0	0.094	3.26×10^5	Adult
C 56	3,750	376	37.3	0.108	3.40×10^5	Adult
C 48	2,370	408	40.6	0.117	3.42×10^5	Adult

head-down, and in this animal the difference was between 0.127 (for the tail-down) and 0.094 (for the head-down). One cat out of the fifteen constituting table 1 showed a head-down value of dV/dP double that of the tail-down (0.156 as compared to 0.078). This phenomenon of head-down values of a different magnitude than the tail-down was observed in one out of every five dogs, while in the cat series this single animal represented the only example.

It should be noted that the grouping of cats according to age presents difficulties of determination, even though inspection of the whole animal (teeth, hair, eyes, skin, bones, etc.), is resorted to. In the group of adults some old animals are possibly included but a frank decision of obvious

old age could not be made with certainty. The group of immature or juvenile animals was limited to those whose general size permitted the completion of the experimental procedures; smaller and still younger animals were apparently unable to stand the repeated tiltings in the anesthetized state. Were the series of animals to be extended beyond the numbers here reported, a gradual transition from one group to another would unquestionably be shown, as indicated by certain overlaps in the values of the coefficient of elasticity in the cats recorded in table 1.

It should be stated that table 1 includes all the cats used for these tilting experiments with four exceptions which for one reason or another were excluded from the tabulation. Thus, cat C 7 was not included as the animal had an outspoken respiratory infection and required artificial respiration throughout the period of experimentation. The other three cats (C 41, 49 and 58) were excluded because in each case only one tilting with manometers other than that of 1 mm. bore was made; these single tiltings were promptly followed by the death of the animal. It seems important to account for the exclusion of data from these four cats as the constancy in value of the coefficient of elasticity is definite in the fifteen cats in which the experimental procedures permitted gathering of adequate data for the determination of the value of the fraction dV/dP and of the total volume V. Only one of the animals (C 50) recorded in table 1 presents a coefficient of elasticity which quite obviously should place the animal in another age-group. In this case an animal classified as adult would fall, as far as the determined coefficient of elasticity is concerned, in the upper end of the group of young adults.

As a matter of record it seems of interest to include here the average pressure-alterations of the cerebrospinal fluid, as recorded by the 1 mm. open-end manometer on vertical head-down and tail-down tiltings, in order to give some idea of the protection of the central nervous system afforded by the bony coverings of the cranium and vertebral arches, against the full effects of atmospheric pressure. Report (2), (3) has been made that in dogs with an average spinal length (occiput to last lumbar spine) of 400 mm., vertical head-down tiltings gave an average increase of 104.9 mm. in the pressure of the cerebrospinal fluid (measured in the occipital region) while vertical tail-down tiltings were followed by an average decrease of 74.3 mm. Data obtained from similar tiltings are now in hand for twenty cats of different sizes and ages; the spinal length in this series averaged 349 mm. with extremes of 322 mm. and 408 mm. Head-down tiltings (61 carried out on the 20 animals) gave an average increase of 123 mm. in the pressure of the cerebrospinal fluid with highest reading of 171 mm. and lowest of 69 mm. The contrariwise tiltings (43 in number, performed on the same 20 cats) afforded an average decrease of 104 mm., with extremes of 151 mm. and 69 mm. The positional pressure-alterations of the cerebro-

spinal fluid are therefore of greater magnitude, in respect to spinal length, in the cat than in the dog; both of these laboratory mammals however show almost the same difference in reaction to the two vertical tiltings, the head-down pressure-change being approximately one-fourth greater.

Macaques. Ten catarrhine monkeys, all macaques, were included in this series; of these, four were Pithecus rhesus and six, the closely allied Pithecus sinicus. The experimental procedures were successfully carried out in all ten, so that no animals were excluded because of technical failures. Unfortunately all of the rhesus macaques were immature, juvenile animals, but the closeness of the two species makes inclusion of the ten animals in one series wholly permissible.

TABLE 2

Derivation of $\frac{dV}{dP}$ *and E for macaque C 30*

MANOMETER	HEAD-DOWN					TAIL-DOWN				
	Pressure-change, C.S.F.	Difference in pressure-change	Volume dislocated	Difference in volume dislocated	$\frac{dV}{dP}$	Pressure-change, C.S.F.	Difference in pressure-change	Volume dislocated	Difference in volume dislocated	$\frac{dV}{dP}$
mm.	*cm.*	*cm.*	*cc.*	*cc.*		*cm.*	*cm.*	*cc.*	*cc.*	
1	14.5		0.252			8.0		0.139		
4	10.8	3.7	1.048	0.796	0.215	6.0	2.0	0.582	0.443	0.221
6	7.1	7.4	1.874	1.622	0.219	4.0	4.0	1.056	0.917	0.229
8	4.7	9.8	2.397	2.145	0.219	2.6	5.4	1.326	1.187	0.219
10	3.6	10.9	2.565	2.313	0.212	2.0	6.0	1.425	1.286	0.214
Average					0.216					0.221

Average $\frac{dV}{dP} = 0.218$. Intradural volume = 82.6 cc. $E = V\frac{dP}{dV} = V/\frac{dV}{dP} = 3.74 \times 10^5$ dynes per cm.^{2} (In C.G.S. units, dP = height in centimeters × acceleration of gravity (980) × density (1.006). Therefore $E = \frac{82.6}{0.218} \times 980 \times 1.006 = 3.74 \times 10^5$ dynes per cm.^{2}).

One of the most important points at issue in this problem on the macaque was the value of the fraction dV/dP for the two types of vertical tilting, head-down and tail-down. The macaque, being a primate living for a large proportion of its life in the erect posture, should give an indication as to whether a specialized mechanism exists for the protection of the nervous system against postural hydrostatic effects of the fluid column within cerebral ventricles and subarachnoid space. In this series of ten animals, eight yielded results which showed a striking similarity in values between the fraction dV/dP derived on head-down tiltings and that on tail-down tiltings. Table 2 shows this identity of values for the two fractions; it

indicates the method of derivation of the fraction as well as its constancy in the repeated tiltings when the experimental conditions are adequate. One Pithecus sinicus (C 22) gave values of the fraction dV/dP for the head-down tiltings of more than double the magnitude of that for the contrariwise tiltings. A second bonnet macaque (C 26) yielded data which made the calculation of the tail-down fraction somewhat more than half that for the head-down tiltings. In each of these two cases, however, the head-down tiltings were of the approximate magnitude obtained in other animals for the two types of tiltings, and the head-down values of the fraction were therefore employed for the determination of the coefficient of elasticity. The occurrence of this phenomenon in two out of ten macaques may be compared to its occurrence in four out of twenty dogs and in one cat out of fifteen.

TABLE 3

Coefficient of elasticity of the dural sac and its contents in macaques

EXPERIMENT NUMBER	SPECIES	WEIGHT	SPINAL LENGTH	INTRADURAL CONTENTS	$\frac{dV}{dP}$	COEFFICIENT OF ELASTICITY	AGE-GROUP	PROBABLE AGE
		grams	*mm.*	*cc.*		*dynes per cm.²*		
C 62	P. rhesus	2,150	242	80.4	0.154	5.15×10^5	Juvenile	21 mos.
C 64	P. rhesus	3,200	244	93.4	0.182	5.06×10^5	Juvenile	21 mos.
C 63	P. rhesus	2,285	234	74.8	0.154	4.79×10^5	Juvenile	31 mos.
C 65	P. rhesus	3,640	282	83.7	0.198	4.17×10^5	Juvenile	34 mos.
C 52	P. sinicus	3,050	260	74.9	0.171	4.32×10^5	Juvenile	40 mos.
C 55	P. sinicus	2,860	256	75.0	0.166	4.46×10^5	Juvenile	43 mos.
C 26	P. sinicus	3,900	312	81.7	0.197	4.09×10^5	Adult	63 mos.
C 34	P. sinicus	2,750	260	69.1	0.164	4.15×10^5	Adult	66 mos.
C 22	P. sinicus	6,250	348	92.0	0.228	3.98×10^5	Adult	8 yrs.
C 30	P. sinicus	5,200	325	82.6	0.218	3.74×10^5	Old	13 yrs.

With these exceptions, the values of the fraction dV/dP were of the same magnitude in the macaque whether calculated from the data of head-down tiltings or of tail-down, though the head-down values in the majority of animals were in slight excess of those from the opposite tiltings. The averages obtained from the two types of positional change from the horizontal were 0.180 for the head-down tiltings and 0.174 for the tail-down—a finding quite comparable to those in the cat and dog. Seven of the ten macaques showed this phenomenon but the other three animals yielded tail-down fractions dV/dP which were somewhat greater than the head-down derivations.

The determinations of the coefficient of elasticity for the macaques are given in table 3. The weights of the animals ranged from 2150 grams to 6250 grams, while the fraction dV/dP yielded its smallest value, 0.154,

in the youngest animal and its highest value, 0.228, in the heaviest (not the oldest) animal of the series. The coefficient of elasticity varied between 5.15×10^5 dynes per cm.^{2} in the most juvenile of the monkeys to 3.74×10^5 dynes per cm.^{2} in the oldest. Again, in these macaques as in the dogs and cats, there was no fixed relationship between body weight, spinal length, intradural contents, and the coefficient of elasticity.

The animals in table 3 are set down in order of age, as determined for the writers by Dr. Adolph H. Schultz, Associate Professor of Physical Anthropology in this institution. The decision as to relative ages was made on the basis of dentition (dental age), skull length and closure of cranial sutures; a maximum error of one-seventh in either direction may be applied to the ages recorded. Certain of the macaques are, as judged by the criteria employed, of almost identical age, particularly in the juvenile or immature group. Thus, animals C 62 and C 64 are both set down as of 21 months' age; the application of the maximum error of one-seventh might make either of these animals the older. Further application of the possible error would make some slight changes in the age-order of the animals in the table, but the order recorded represents the best judgment of age. The separation of the juvenile from the adult animals is in some ways an arbitrary one but it follows accepted anthropological usage. In all these determinations of age the greatest reliance has been placed upon the dental formula.

Analysis of the data in table 3 makes it apparent that the younger animals have coefficients of elasticity considerably higher than the adult and old animals. The coefficients of elasticity in the macaque series follow the age-grouping quite well, though one animal (C 65) classed as a juvenile shows a coefficient of elasticity which would place it among the adult animals. That one animal should fall out of series with the others in the experimental group is not surprising: rather is it surprising that such constancy in the coefficient of elasticity should be exhibited.

The experiments performed on this group of macaques gave information as to the extent of the pressure-changes in the cerebrospinal fluid, on tilting from the horizontal to the two vertical positions, when determined in the customary open-end 1 mm. manometer in the occipital region. Thirty-six tiltings to the head-down position in the ten monkeys gave an average pressure-increase of 106 mm. in the occipital cerebrospinal fluid, with 152 mm. recorded as the largest and 72 mm. as the smallest increase. The tail-down tiltings gave an average pressure-decrease of 80 mm. with extremes of 122 and 60 mm. The average spinal length (occiput to last lumbar spine) was 276 mm. in this group of ten macaques, the longest measuring 348 mm. and the shortest 234. These average pressure-changes in the occipital cerebrospinal fluid, in their relation to the average spinal lengths, may be directly compared to the similar pressure-alterations recorded for the dogs and cats.

DISCUSSION. The significance of the general coefficient of elasticity of the dural sac and its contents has been discussed in the previous reports of experiments on dogs (1), (4) and need not be repeated here. The general coefficient of elasticity of the cat is slightly lower than that of the dog, and shows similar age-changes. The cats may be taken to be of the same age-groups as the dogs but no obviously old cats were included. In the macaque series, the four Pithecus rhesus were unquestionably younger than any of the cats or dogs experimented upon while the six Pithecus sinicus represented all of the age-groups included in the dog and cat series and can therefore be directly compared to them. Taking the two juvenile specimens of the bonnet macaque for comparison with the immature dogs and cats, the average coefficient of elasticity of the dural sac and its contents is of the same magnitude in the dog and macaque, slightly lower in the cats. Similarly, the coefficients of elasticity in the young adults, adults and old animals are of the same values in the macaques and dogs, slightly less in the cats. All three of these mammals show in decisive fashion a gradual decrease of the coefficient of elasticity in dynes per cm.^{2} (i.e., decrease in resistance to deformation) as one progresses from the juvenile into the adult and old animals. The occurrence of this phenomenon in these three laboratory animals suggests the strong possibility that it is of general biological significance.

Apart from any importance these observations may have in permitting calculations of unknown factors by substitution in the general physical formula employed, the experiments on macaques seem clear-cut in demonstrating that in this primate the general physiological mechanisms of pressure-adjustments about the central nervous system, in response to positional change, are not essentially different from those of the cat and dog. These two mammals may be taken to be typical four-footed animals in which the central nervous system is habitually carried in the horizontal position with only slight elevation of the head. The macaque, on the other hand, as has been pointed out, is an essentially erect animal, resting and usually sleeping in the vertical position though progression remains horizontal and four-footed. Had the assumption of the erect posture been accompanied by the development of protective agencies securing the nervous system against hydrostatic effects of the continuous column of the cerebrospinal fluid, difference in the reactions to tilting from the horizontal to the two vertical positions (head-down, tail-down) would be expected. First of all, the pressure-changes in the cerebrospinal fluid on such positional alterations would have been different in magnitude from those of the dog and cat. Yet the macaque showed on tilting to the head-down position an average pressure-increase of 106 mm. in the occipital cerebrospinal fluid and an average decrease of 80 mm. on the opposite positional change. With a spinal length considerably less than that of the dogs and cats used

(276 mm. as compared to 400 mm. and 349 mm. respectively), the average pressure-alterations in the macaque are the same as in the dog and slightly less than in the cat. Again, the general coefficient of elasticity (as calculated from the values of the fraction dV/dP) was found to be, in eight out of ten macaques, of the same magnitude whether derived from data furnished by head-down or tail-down tiltings. The exceptional animals which yielded coefficients of elasticity of almost double the magnitude from head-down tiltings as from the contrariwise positional changes, are encountered in the same proportion in dogs (one out of five) as in the macaque.

These two general characteristics of the reactions to tilting in the macaque indicate that the adoption of the erect posture has not been accompanied by the development of new physiological mechanisms for the protection of the nervous system against the hydrostatic pressures in the cerebrospinal fluid which the vertical position imposes upon the animal. Such protection as the bony coverings of the nervous system afford against the full effects of atmospheric pressure seems to be of the same degree in the macaque, the dog and the cat. The elastic elements within the bony cranio-vertebral canal also have coefficients of elasticity of the same general order of magnitude in the three mammals used: the coefficients for the macaque and dog are quite similar while that of the cat is slightly less.

It should be pointed out that these experiments have all been carried out on anesthetized animals and that there is of course a possibility of modification of the reactions due to this factor. As has been noted elsewhere (2), (3), such observations as have been made in unanesthetized man with lumbar pressures of the cerebrospinal fluid taken in the prone and sitting positions, indicate that the positional pressure-changes in this fluid are of the same relative magnitude in the unanesthetized as in the anesthetized state. It seems fair to conclude therefore that the data obtained from anesthetized mammals in these experiments may be accepted as portraying the animal's physiological reactions to positional change.

SUMMARY

The coefficients of elasticity of the dural sac and its contents have been determined in a series of 15 cats and 10 macaques, by application of the physical formula $E = dP \Big/ \frac{dV}{V}$. Deriving the fraction dV/dP from tilting experiments with measurement of the differences in pressure of the cerebrospinal fluid and in volume of fluid dislocated into or from manometers of various bores, the coefficients of elasticity were found to have the following values: in the macaques, from 5.15×10^5 dynes per cm.^{2} in a very young juvenile to 3.74×10^5 dynes per cm.^{2} in an old animal; in the cats, from 3.86×10^5 dynes per cm.^{2} in an immature to 3.26×10^5 dynes per cm.^{2} in an adult animal. The series showed a gradual decrease in the coeffi-

cients of elasticity from the immature to the old animals; in the various age-groups a fair degree of constancy in magnitude of the coefficients of elasticity was noted.

BIBLIOGRAPHY

(1) FLEXNER, L. B., J. H. CLARK AND L. H. WEED. 1932. This Journal, ci, 292.
(2) WEED, L. H. 1929. Intracranial pressure in health and disease Chap. iii, p. 25 (Assoc. Research Nerv. & Ment. Dis., vol. viii, Baltimore).
(3) WEED, L. H. 1929. Arch. Surgery, xviii, 1049.
(4) WEED, L. H., L. B. FLEXNER AND J. H. CLARK. 1932. This Journal, c, 246.

ACTION POTENTIALS FROM SINGLE MUSCLE FIBERS

S. GELFAN AND G. H. BISHOP

From the Department of Physiology and Pharmacology, University of Alberta, Edmonton, Canada, and the Department of Ophthalmology, Washington University, and Laboratory of Applied Physiology, Oscar Johnson Institute, St. Louis, Mo.

Received for publication June 4, 1932

Adrian (1922) found, after stimulating small groups of skeletal fibers by the pore electrode, that the action potentials from these fibers varied in a discontinuous steplike fashion as the strength of stimulus was altered. The action potentials were proportional to the number of fibers activated, and apparently did not vary in a single fiber. Adrian therefore suggested that the all-or-none behavior of a muscle fiber is determined early in the chain of events from stimulus to response.

Since it has been demonstrated with microstimulation that normal single skeletal fibers can respond submaximally, depending upon the strength of stimulus (Gelfan, 1930, 1931; Pratt, 1930; Brown and Sichel, 1930),[1] the following question is raised: Is there an action potential present during a submaximal contraction of a single fiber, and does it vary with the degree of response of the single element?

Gelfan and Gerard (1930) showed that the gradations in contraction of a single fiber are not continuous from one end of the fiber to the other. The progressive spreading of the localized contraction from the point of stimulation, as the strength of stimulus is increased, is continuous only to a certain degree, after which a further increase in stimulus intensity elicits a complete and maximal response. These authors suggested that the submaximal responses may be due to a direct stimulation of some of the contractile units (sarcomers) by currents that are unable to initiate the conducted response. If that were true, an action potential might not be present during the submaximal contractions, since conduction of the response is absent. This would also answer the above-raised question. The following experiments were therefore undertaken to determine whether an action potential is present in single fiber during partial or submaximal contraction.

Diphasic action potentials were recorded by means of the cathode ray oscillograph both from sartorius and from the muscle fibers of the retrolingual membrane, stimulated adequately for conduction to result, at a

[1] See also Fischl and Kahn, 1928, and Hintner, 1930.

sensitivity of the apparatus of 60 mm./mv., using microelectrodes, to establish a normal procedure for this technic. Such action potentials were in all respects identical with those recorded by Bishop and Gilson (1929) from the frog sartorius at threshold at 50 mm./mv. sensitivity, using fine steel wires as electrodes, the lead electrodes being placed accurately on the active fibers. Our present observations under the microscope indicate that such threshold responses were also due to single-fiber responses in the sartorius. In our present work, when the stimulus strength was increased, the action potentials increased by unit steps equal to the initial threshold response in amplitude. In the sartorius, the lead electrodes could be placed at first far enough apart to separate the two phases of the action potential. They were then brought closer together to ascertain whether, when the electrodes were separated by the distance that is permitted by the relatively short fibers of the retrolingual membrane, an action potential could be recorded at all from diphasic leads. Since the available length of fiber here is about 3 mm., an impulse conducting $2\frac{1}{2}$ m./sec. would give a record in which the two phases were superposed and of opposite sign after about 0.001 second, and at this time the first phase has reached only a fraction of its maximal value (fig. 3).

It was found that by resorting to a shunting device such reduction in amplitude of the diphasic record could be largely obviated. It was necessary in any case to arrange the electrodes and the muscle in the form of a Wheatstone bridge, to avoid the large shock artefact appearing when a stimulus of the order of one volt was applied at a distance of a fraction of a millimeter from one lead, with apparatus recording at the rate of 1 volt = 60 meters. The electrodes were first arranged with respect to the fiber (fig. 1) so that the resistance pathways in the tissue itself formed such a bridge, except that the balancing of the bridge was an extremely delicate procedure in the first place, and in the second, the balance changed both with movement of the fiber and with change of strength of stimulus. Two other bridge arms were therefore inserted, consisting of variable graphite-line resistances of the same order of magnitude as the stimulating cathode (100,000 ohms) arranged parallel to the stimulating electrodes as in figure 2. After initial approximate balance by movement of electrode 4, further compensation could be made as the stimulus was raised toward threshold by adjusting R5 and R6.

The virtues of this arrangement lie in the following secondary details: first, making the main bridge of the tissue itself and of the electrode resistances allowed the bridge to be approximately balanced for polarization at the same setting as for resistance, and without external bridge capacities. This was somewhat interfered with by the electrodes being of different sizes. Secondly, since electrodes 3 and 4 were grounded through resistances 5 and 6 about as effectively as was electrode 2, and since electrode 4

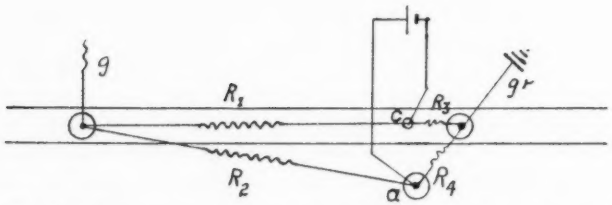


Fig. 1. Arrangement of electrodes on single muscle fiber for recording action potentials in response to stimulation. *g*, grid electrode; *gr*, ground electrode, leading to the amplifier of the cathode ray oscillograph; *a* and *c*, anode and cathode microelectrodes for stimulating. The tissue resistances between these four electrodes can be considered diagrammatically as the four arms of a Wheatstone bridge. See text.

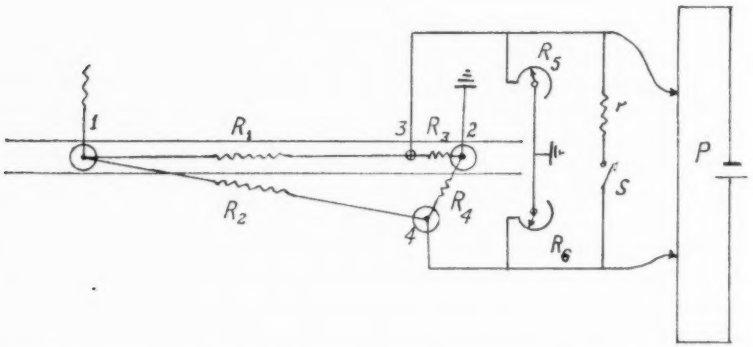


Fig. 2. Diagram as in figure 1 of single muscle fiber with leading and stimulating electrodes, *1* and *2*, and *3* and *4*, respectively, with the stimulating apparatus employed. *P*, potentiometer source of current; *s*, short-circuiting key which is opened to stimulate; *r*, protective resistance in series with *S*, R_5, and R_6, adjustable resistances in parallel with the two arms of the bridge R_3 and R_4, for balancing bridge after approximate balance has been obtained by moving electrode *4*. For effect of this arrangement on the action potential record see text.

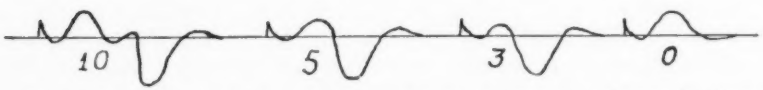


Fig. 3. Diagrams of the conducted potential records from a single muscle fiber at the surface of the frog sartorius, recorded in situ, with 10, 5, and 3 mm. distance between lead elctrodes; and diagram, based on the previous ones, representing what the nonconducted action potential of a single muscle fiber would presumably have looked like if it had arisen under the stimulating cathode, but failed to conduct to the second lead electrode.

especially was a large one of low resistance, the result was a diffuse ground lead from these three electrodes in parallel, which materially reduced the amplitude of the record of the first phase of the action potential as compared to the second, thereby allowing their algebraic sum to be considerable even when superposed. On the other hand, if the second phase had failed to appear by reason of incomplete conduction, the first phase would have been of sufficient height to be clearly visible, as was demonstrated in the sartorius experiments where the leads could be placed far enough apart to separate the two phases in the record. At 3 mm. distance between electrodes 1 and 3, a separation just obtainable in the retrolingual membrane, a diphasic record showed a brief but detectable initial negative deflection followed by a much greater positive one (fig. 3). The whole might then have been interpreted as a single second phase were it not for the controls at greater separation.

After initial trials with condenser charges, galvanic currents from a potentiometer were used to stimulate for greater ease of compensation in the bridge. The potentiometer of 1,000 steps was built to have its capacity to ground about symmetrical with respect to the output leads (fig. 2). Battery current was allowed to flow continuously, the current through the bridge being turned on by breaking the short circuit S, a protecting resistance r of 25 ohms being insignificant as compared with bridge resistance. The interval of the opening of the short circuit could be varied from a small fraction of a thousandth of a second to about twenty thousandths by means of a rotating circuit breaker, or by a hand tap key for longer durations.

The electrode-tissue resistance was measured for high frequency and for direct currents (Bishop, 1929) to determine what complications polarization would introduce. The polarizable resistance of 25 mu electrodes proved to be several times their ohmic resistance, and was only partially reduced by plating with silver—silver chloride. The result is that even with a low constant voltage the current is at a maximum at first, then falls to a small fraction of this, in a time of the order of a sigma. The start of any galvanic current thus resembles a condenser charge or an induction shock in form, and for the rest of the current duration its effect may be insignificant. In spite of this, careful adjustment of threshold enabled us to obtain a few records late in the duration of the galvanic flow, after the initial distortion due to polarization had subsided. Such records were always diphasic, indicating all-or-none conduction, but this might have been due to the fact that current sufficient to stimulate at all would, if allowed to flow longer, mount to a stimulus adequate for complete conduction. If the current was now shortened in duration until it barely stimulated, the record was diphasic until both phases disappeared together, but then the break of the current with its concomitant distortion tended

to obscure the record. Shortening the duration of threshold currents usually required, however, no increase of strength for threshold until the end of the current fell within the polarization period of the start, indicating again that polarization promptly reduced the current led from a constant applied voltage to an ineffective value. Therefore the currents were usually shortened to a sigma or less, and the threshold regulated by the voltage, the stimuli then resembling rather long induction shocks, with their tails cut off.

One other complication entered. Many muscles would not respond to a stimulus below 1.3 volt with any duration (although some responded at less than 0.5 volt). At about 1.3 volt applied potential a sudden increase took place in the record of the stimulus distortion, and if the current was allowed to flow, bubbles arose from the electrodes. Apparently at about the dissociation potential difference for water an abrupt change takes place in the polarization phenomena at the electrodes, even before visible gas is evolved, and the distortion of the record associated with this is so large as to render further procedure impracticable. Increasing the size of the electrodes appeared to decrease somewhat the voltage necessary to stimulate, but this was limited by the fact that above 30 or 40 mu all threshold responses are conducted in an all-or-none manner, the voltage range for incomplete responses decreasing with increase in size of the stimulating cathode. An abrupt change in the character of the shock distortion in nerve stimulation is a common occurrence, even with induction shocks or condenser charges, and may presumably also be assigned to the passage of enough current across the electrodes, or perhaps across the tissue membranes, to initiate gas polarization by the dissociation of water.

In the muscle fibers of the retrolingual membrane of the green frog, we were unable to detect any record of excitation potential similar to a normal muscle action potential, from an incompletely conducted, i.e., submaximal, response. In some cases a difference of a few millivolts in threshold determined consistently whether a diphasic action potential or none at all was elicited. In others, the threshold rose slowly but progressively, even though stimulated only about once per two seconds. It might be argued here that with a small response of the fiber, only in the region of the stimulus, a weakened single first phase would not have been detectable at the sensitivity employed. However, under conditions where less than one per cent change in stimulus determined the character of the response, it seems reasonable to suppose that an impulse localized under the electrode, and due to a stimulus just subthreshold for conduction, would not be far below the conducted impulse in intensity, if it were otherwise of the same character. The fact that the break between the full-sized potential of the conducted response and its apparently total absence in the case of the nonconducted one is so complete and so sharp, even with

accurately graded stimuli, indicates that the two responses are different in type; that is, with respect to the electrical manifestations of response. Aside from the matter of conduction, the *mechanical* responses may be similar.

In two experiments fibers were encountered where marked treppe occurred. With the interrupter running at 2-second intervals the stimulus voltage was raised to just threshold for a conducted response. It could then be gradually lowered by as much as 20 per cent with the fiber responding each time all-or-none. If a few seconds' interval were then allowed without stimulation, the threshold had returned to near its initial value. This degree of treppe, reminiscent of the usual condition in the vertebrate heart, is much greater than that sometimes showing in fatigued nerve. That these muscles were not more than temporarily fatigued was indicated by the fact that the series could be repeated on the same fiber again and again, and by the further fact that the action potential record did not materially fall off during the procedure as it does, parallel to the tension, in normal fatigue. Neither, however, did the potential rise noticeably as the fiber became more irritable. This is a treppe, then, at least in the sense of progressive increase in irritability with activity, if not an increase in response. We do not know whether the contractions altered in tension developed, although all-or-none contractile responses of single fibers as recorded by the mercury droplet method, do exhibit the staircase phenomenon (Pratt and Eisenberger, 1919, fig. 30).

As far as could be judged visually, under the microscope, the duration of the nonconducted responses appeared quite comparable to the conducted ones. It is difficult to determine whether the spread of the submaximal contraction in the single fiber, as compared to the extremely localized minute twitch under the electrode, constitutes a *partial* conduction of the contractile wave. Lillie (1929) has pointed out that in the passive iron wire, a fine scratch, that is, activation of only a small surface area, does not evoke the transmission of the response. The very slight spread in this case quickly decrements to zero. In the muscle fiber, however, though the spread of the submaximal response is limited, it is considerable. If the response is a partially conducted one, it is reasonable to expect, from the above considerations, that the presence of a decremented action potential would have been detected in our experiments. On the other hand, if the localized responses are due to a direct activation of the contractile elements of the fiber, without at the same time initiating the propagated response, it might be assumed, as Gelfan and Gerard (1930) have done, that the spread of the localized response is due to the spread of the localized stimulus.

In two experiments with the retrolingual membrane, the fibers failed to give a conducted response at less than $4\frac{1}{2}$ volts applied potential, with any

duration of stimulus. They gave good nonconducted responses at somewhat less than this, but not with short durations of stimulation of the order of sigma. Stimuli of longer duration were therefore applied by means of a tap key worked by hand. It was then observed that the responses lasted as long as the stimulus, not as a tetanus, but as a nonconducted *contracture*. These fibers thus differed from normal ones not chiefly in ease of eliciting nonconducted responses, but in the high threshold for all-or-none response, such that the range of stimulus strength over which the incomplete response could be obtained was extremely wide.

This is obviously the idiopathic galvanic contracture characteristic of muscle in poor condition or of invertebrate muscles. Now maintaining the strength of stimulation below that required for conducted responses, different durations of current were applied. Here all succeeding responses must have been local ones and of the character of contracture rather than of propagated impulses. The object was to determine whether indubitable contractures could relax so rapidly after a brief stimulus as to be indistinguishable in duration from twitches, conducted or not, such as had been observed in normal muscles. Visual observation under the microscope would at times impress the observer that these contractures were more sluggish and had a longer relaxation period, as compared to the localized response of a normal muscle fiber. The difference in duration however, if any, between brief contracture responses and conducted ones, could not be definitely determined by visual inspection. This aspect of the problem will be further investigated photographically.

We may, therefore, provisionally conclude that the submaximal contractions of a single skeletal fiber, what have here been termed nonconducted or partially conducted responses, differ from completely conducted ones in lacking a normal action potential. Since the duration and other characteristics of these contractions cannot be definitely distinguished, by the means so far employed, from responses that are short contractures, it is conceivable that they may be of the nature of brief contractures. Their twitchlike brevity is permitted by the briefness of the stimulus, whether this be an induction shock or galvanic current promptly blocked by the polarization of the small electrodes that must be employed. It is possible, when stimulated by a current of the order of duration of the muscle action current itself, that a local contracture may take place of the order of duration of the normal muscle all-or-none contraction. Such a viewpoint would not presume any fundamental difference, so far as may be detected by the methods employed, between a muscle contraction and a muscle contracture, except that one appears to be initiated by the muscle action current, the other by current from a battery. This is consistent with the viewpoint take by Gasser, in defining contracture (1930, p. 36), that "only conduction of the mechanical response and a wavelike action

potential are missing." How significant this slight difference might be for an interpretation of the physiological action of muscle, however, only further investigation may decide.

SUMMARY

1. A method is described that permits cathode ray oscillograph measurements of action potentials from single muscle fibers when the latter are activated by microelectrodes to give conducted responses.

2. In the single muscle fiber of the retrolingual membrane, no action potential could be detected during submaximal contraction.

BIBLIOGRAPHY

ADRIAN, E. D. 1922. Arch. Neér de Physiol., vii, 330.
BISHOP, G. H. AND A. S. GILSON, JR. 1929. This Journal, lxxxix, 135.
BISHOP, G. H. 1929. This Journal, lxxxix, 618.
BROWN, D. E. S. AND F. J. M. SICHEL. 1930. Science, lxxii, 17.
FISCHL, E. AND R. H. KAHN. 1928. Pflüger's Arch., ccxix, 33.
GASSER, H. S. 1930. Physiol. Rev., x, 35.
GELFAN, S. 1930. This Journal, xciii, 1.
1931. Ibid., xcvi, 16.
GELFAN, S. AND R. W. GERARD. 1930. This Journal, xcv, 412.
HINTNER, H. 1930. Pflüger's Arch., cciv, 608.
LILLIE, R. S. 1929. Journ. Gen. Physiol., xiii, 1.
PRATT, F. H. AND J. P. EISENBERGER. 1919. This Journal, xlix, 1.
PRATT, F. H. 1930. This Journal, xciii, 11, 680.

THE BLUE EXCITATION CURVE OF DICHROMATS

ELLIS FREEMAN AND W. F. HAMILTON

From the Department of Psychology, and Department of Physiology and Pharmacology, University of Louisville

Received for publication June 4, 1932

This investigation aims to find the characteristics of dichromatic or color-blind vision which may be a check upon the data of the trichromatic functions of the normal eye described in an earlier paper (1). The method of making the matches to spectral monochromes and the apparatus were the same as those employed for the normal.

In table 1 the first pair of subjects are protanopic, and are represented by the lower curve in figure 1 for matches between 660 and 500. The second pair are deuteranopic, and are represented by the upper curve for the same range. All four subjects, since they have identical blue functions, are represented by the same curve for matches between 517 and 460.

In our paper on normals the results differed from the classical data in that our mixtures were as saturated as the monochromes which they matched as far down as 517. Classically an obvious loss of saturation has been described in mixtures which match monochromes up to 570 or higher, and the blue excitation curve has been extended deep into the long-wave region of the spectrum to represent this phenomenon. In attempting to account for the difference between our results and those of König, Abney, Wright, Guild, and others, we have found a sufficient general explanation in the state of adaptation of the eye. Without exception the experimental procedures suggest that, when the loss of saturation in this region occurred, the subject was adapted to an intensity below the intensity of the stimulus. In our work, where the loss did not occur, the subject was adapted to brightness comparable to that of the stimulus,—the condition of all ordinary useful vision. Thus our work on normals pointed to the likelihood that the loss in the observations of others was due to desaturating scotopic factors introduced by dark-adaptation.[1] It also

[1] Whereas light-adaptation and the other factors discussed in our previous paper may be a complete explanation of the divergence between the classical and our results, it should be further noted, for what it may be worth, that tentative observations designed to elucidate the rôle of field size have produced desaturations even in the presence of light-adaptation. These occur simply when the fields are viewed through an ordinary scale-reading telescope placed at the eye-piece of the original apparatus. With the telescope so placed, desaturation seems to be independent of field size, intensity, and stray light.

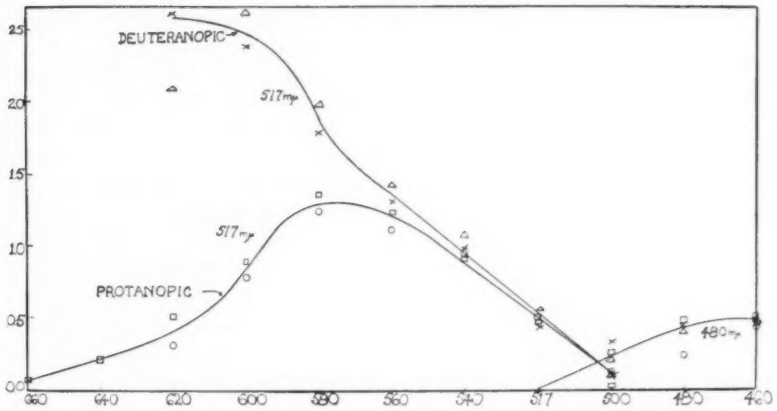


Fig. 1. Excitation (spectral color-mixture) curves of two types of dichromats. Wave-length in mμ.

TABLE 1

Showing spectral color-mixture matches by two types of color-blind subjects

The first type (2 subjects) possessing a protanopic curve, and the second type (2 subjects) possessing a deuteranopic curve. The values are given in millimeters of slit width for each of the primaries required to match the monochrome.

SYMBOL OF SUBJECT	MONOCHROME MATCHED, mμ	PRIMARIES		SYMBOL OF SUBJECT	MONOCHROME MATCHED, mμ	PRIMARIES	
		517 mμ	480 mμ			517 mμ	480 mμ
Square	460		0.48	Triangle	460		0.48
	480		0.46		480		0.44
	500	0.01	0.24		500	0.10	0.28
	520	0.47			520	0.50	
	540	0.91			540	1.07	
	560	1.23			560	1.42	
	580	1.36			580	1.97	
	600	0.89			600	2.61	
	620	0.51			620	2.09	
	640	0.21			670	2.21	
	660	0.07					
	670	0.05					
Circle	460		0.42	Cross	460		0.48
	480		0.23		480		0.43
	500	0.12	0.27		500	0.11	0.32
	520	0.49			520	0.45	
	540	0.94			540	0.97	
	560	1.11			560	1.30	
	580	1.24			580	1.79	
	600	0.78			600	2.38	
	620	0.31			620	2.61	

indicated that a set of excitation curves representing photopic vision possesses a blue curve that falls to 0 in the region of 517.

Since the characteristics of partial color-blindness arise from the apparent absence of either the red or the green process, we should expect the corresponding excitation curves to indicate this fact, as they do. The blue curve, on the other hand, is like that of the normal. This conformity of blue in normals and in dichromats offers a simplified means of making a check on the results obtained with the normals.

In making the complex matches of the normals above 517, the remote possibility remained that very small amounts of desaturation in the mixed field had been neglected. The color-blind eye, since it involves fewer variables, offers a simple way of determining the extent of the blue curve. Since we have found the blue curve for the color-blind to coincide with that of the normal, it constitutes an important argument against the remote possibility that the blue curve had not been extended sufficiently far towards the red in the case of the normals.

The greater simplicity of determining the excitation curves of the color-blind lies in the circumstance that matches above the vicinity of 517 can be made with only one primary. Thus the color-blind had no more difficulty in making a "heterochromatic" match than the normal would have in matching two fields of the same wave-length. The fact that this was true when matches were made between 517 and longer wave-lengths shows conclusively that in the region of 517 there is no blue excitation.[2]

Not only is it simpler to determine the excitation curves of the dichromat, but also it is easier to determine his wave-length discrimination curves. Steindler (2) has described the "hue" discrimination curve of the deuteranope as consisting of a single region of low threshold near 500. This has been corroborated by other investigators (3). In contrast with Steindler's complex curve for the protanope, however, a simple curve has been found (3), (4), identical in all respects with the curve of the deuteranope. Evidently, as has been suggested (4), Steindler allowed her subjects to mistake brightness differences for hue differences. The identity of the wave-length discrimination curves for the two types of dichromats is consistent with, and to be expected from, the coalescence of the two types of "long" excitation curves of figure 1 in the vicinity of and below 517. Of course, the red and green excitation curves of the normal show no such tendency to come together here (1).

The fact that wave-length discrimination is confined to a restricted region in the blue-green and that it is entirely lacking above the region of 517, in the color-blind, justifies still further the restriction of the blue curve to below the region of 517 in the normal.

[2] This is subject to the assumption that the blue curve cannot be proportional to both the protanopic and deuteranopic "long" curves at all wave-lengths (1).

SUMMARY

Considerations of color-mixture and of wave-length discrimination in the two types of dichromats indicate, as do the data for the normal eye, that the blue excitation curve should not extend above the region of 517.

BIBLIOGRAPHY

(1) HAMILTON W. F. AND E. FREEMAN. Journ. Optical Soc. America, June 1932.
(2) STEINDLER. Sitzungsb. d. Wiener Akad., 1906, cxv, ii A, 39.
(3) In press.
(4) LAURENS, H. AND W. F. HAMILTON. This Journal, lxv, 1923, 547.

CATALEPSY CAUSED BY LESIONS BETWEEN THE MAMMILLARY BODIES AND THIRD NERVE IN THE CAT

S. W. RANSON AND W. R. INGRAM

From the Institute of Neurology, Northwestern University Medical School

Received for publication June 6, 1932

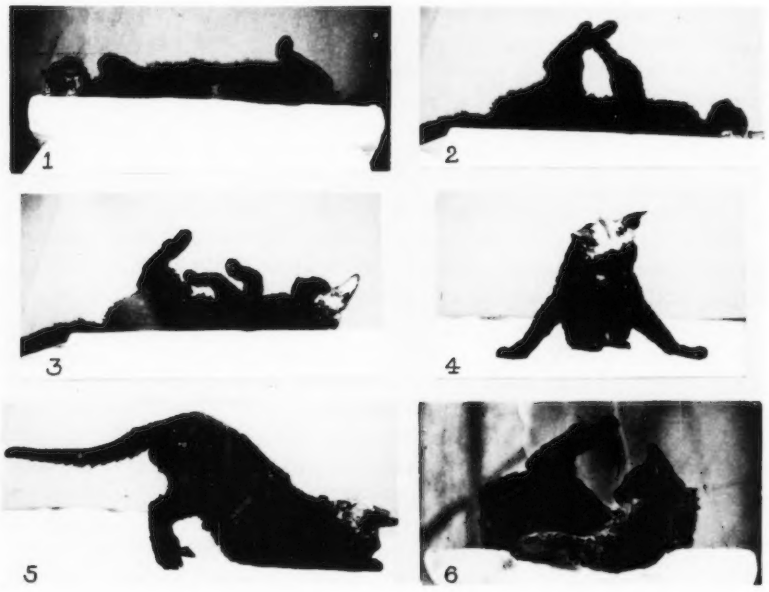
With the Horsley-Clarke apparatus and a bipolar needle electrode we have placed small bilateral electrolytic lesions in the region between the caudal border of the mammillary bodies and the rostral fibers of the third nerve. This work was undertaken as an extension of an investigation of the functional disturbances caused by the destruction of the red nuclei, and the technique employed has been fully discussed in the report of those experiments (Ingram and Ranson, 1932). In the course of those experiments it had been noted that cats, in which the lesions had extended some distance rostral to the third nerve, exhibited an apathy and inertia quite striking when compared with those in which the lesions did not extend so far forward.

OBSERVATIONS. The investigation was renewed in order to determine the effect of lesions situated just rostral to both red nuclei. Many of the cats in this series were very lethargic and required rather strong stimuli to wake them up. They would maintain for long periods of time any posture in which they happened to be placed. Their behavior was so different from that of cats with lesions more caudally placed in the red nuclei that our attention was at once directed to their cataleptic state.

In five of the eight cataleptic cats the lesions had been made under ether anesthesia and without aseptic precautions. Within three or four hours they had recovered from the anesthetic sufficiently to permit examination. They were examined again six hours after the operation and again at the end of twenty-four hours, when they were killed to prevent the results being complicated by infection. Within three or four hours all of these cats could stand and walk. They were, however, disinclined to walk and would never take more than a few steps at a time and would come to rest as soon as they were permitted to do so. If when they were lying flat on their side the tail was pinched, they could readily get up into a standing position. But if left undisturbed they would lie quietly and apparently asleep.

They exhibited an increased muscle tonus of an extremely plastic type, by virtue of which they would assume and maintain any posture in which

it pleased the experimenter to mold them. The plasticity could be especially well demonstrated when the cats were resting on their backs in a shallow trough (figs. 1 and 2). If the limbs were passively flexed they remained flexed. If they were passively extended they remained extended indefinitely, and considerable force was required to return them again to the flexed position. In spite of this marked resistance to flexion, which was as great as in the best decerebrate preparations, the limbs when they



Figs. 1, 2, 3, 4, and 5. Cataleptic cat three days after bilateral electrolytic lesions had been made caudal to the mammillary body. On the first day this cat would take and hold the position shown for another cat in figure 6, but on the third day it would only hold its head up as shown in figure 3.

Fig. 6. Cataleptic cat four hours after bilateral electrolytic lesions had been made caudal to the mammillary body.

had once been passively flexed exhibited no tendency to extend themselves. These cats differed from decerebrate preparations, whether made by the transection or anemic methods, for according to our experience the limbs of a decerebrate cat showing a comparable degree of resistance to passive flexion will not remain flexed for any considerable period of time but will always return gradually to the fully extended position. In this respect these animals were much more plastic than any decerebrate preparations which we have examined.

This plasticity also involved the muscles of the neck, trunk, and tail. If the head and pelvis were raised from the trough they would remain elevated for a time (figs. 3 and 6), but this posture was one which could not be maintained for more than a minute. The head and pelvis would gradually yield to gravity and sink back into the trough.

The animals could be molded in various standing positions (figs. 4 and 5) and would maintain them for long periods. They have been observed to stand in these absurd postures for as much as fifteen minutes at a time. A stimulus, such as pinching the tail would, however, wake them up and cause them to assume again a normal posture and take a few steps, after which, if left alone, they would apparently go to sleep again in the standing position.

So far as could be judged by their behavior these animals were very drowsy and slept most of the time. They seemed to sleep equally well on their backs in the trough or when standing on their feet. They paid no attention to ordinary stimuli such as sounds or stroking of the hair but could be aroused by pinching the tail. The threshold for painful stimuli was raised so that stimuli, which would ordinarily obtain a prompt and decisive response, would have to be repeated or increased in intensity to arouse the animal. Apparently their readiness to maintain unusual and rather uncomfortable postures was directly related to this somnolence, and the paucity of voluntary movements was an important factor in the ease with which plasticity would be demonstrated.

When lying on their backs in the trough with limbs extended or when supported in a canvas hammock with the limbs pendant, the cats offered considerable resistance to passive flexion of all four legs. This could readily be demonstrated whether the flexing force was applied above the wrist or ankle or whether it was applied to the pads of the toes in the position of the positive Stütz reflex. As has already been intimated these cats differed from decerebrate preparations in that when lying on their backs in the trough, there was no tendency for the limbs to take on of themselves an extended position though, when passively extended, they would retain this position with considerable strength. Furthermore, these cataleptic cats differed from decerebrate preparations in their ability, when properly aroused, to get on their feet, stand and walk. If, when lying on their backs in the trough with the limbs extended in the air the trough was tipped so that they fell over on their side, they would wake up, get on their feet, and perhaps take a few steps and then apparently go to sleep again in the standing position. Sometimes under such conditions the contraction of the muscles involved in maintaining a standing position would be gradually overcome by force of gravity; the head would droop until it touched the floor, the limbs would sag and occasionally assume sprawling positions until finally the cat was lying, asleep, often in odd

postures. If the animal went to sleep with the head and fore quarters hanging over the edge of the table it would gradually slip off and fall, waking up only when it struck the floor.

A normal cat will seek out a comfortable spot before lying down to sleep; but our cats exhibited a more or less persistent somnolence which caused them to fall asleep in almost any position. Although they were not comatose, because they could be aroused and would then react in a fairly normal manner, their sleep could not be regarded as normal. It resembled in many respects that seen in patients with encephalitis lethargica.

The cats would eat, if food was held to their mouths, but in quite an automatic, almost reflex manner, without particular interest.

In most instances the pupils were widely dilated indicating an involvement of the Edinger-Westphal nucleus. In one instance both pupils were constricted and in this case the lesion must have been situated rostral to that nucleus. The eyes were usually held closed, which may have indicated a ptosis due to paralysis of the third nerve or a contraction of the orbicularis oculi.

In several cats the lesions were placed under aseptic conditions. Three of these were cataleptic. One remained in this state for seven days but died on the eighth day of pneumonia. Another remained cataleptic for three days. The fourth day no observations were made. On the fifth day it was wide awake and active. The third cat remained cataleptic for only two days after which the somnolence disappeared. In these two animals, after the lethargy had disappeared and they had again become alert and active, the plasticity also disappeared and they could no longer be posed in unusual postures but would again voluntarily assume normal attitudes.

Microscopical sections of the brains from these animals showed that in all cases the lesions were bilateral and were located in the region between the mamillary body and the third nerve. Usually two and sometimes three small lesions had been placed one rostral to another on each side of the midline and were more or less confluent rostrocaudally but not across the midline. This confluence produced in effect a single irregular lesion on each side of the midline about three millimeters long and one-half to one millimeter wide. Although there was great variation in the shape and size of the lesions and in the structures which were destroyed, it may be said in general that the lesions centered around the point where the habenulopeduncular tract approaches the ventral surface of the brain stem before turning caudally toward the interpeduncular nucleus. A study of the sections does not enable us to say what structure it was, the destruction of which was responsible for the cataleptic symptoms. The lesions were too varied in shape and extent. And there is the possibility to be considered that the effect was not produced by the destruction but rather

by the zone of irritation surrounding the lesion. The case is further complicated by the fact that other cats with similar lesions showed no signs of catalepsy. The symptom complex when obtained was, however, so characteristic and differed so markedly from anything which we have seen in a rather extensive experience with lesions in other parts of the cat's brain that we believe we are here dealing with a definite syndrome and that additional experiments will enable us to fix more definitely the location of the lesion, whether destructive or irritative, which is required to produce catalepsy in cats.

Discussion. Lesions made by sticking a knife into the deeper parts of the brain are necessarily too extensive and too poorly localized to have much value in such a problem as this. The figures given by Spiegel and Inaba (1927) indicate that most of their lesions were unilateral and that they were not at all similar to those in our cataleptic cats. Reference should also be made to the experiments of Hess (1931) who was able to induce sleep in cats by electrical stimulation of deeply situated parts of the brain. But, since his figures indicate that this result was obtained from such widely separate and functionally diverse regions as the septum between the anterior horns of the two lateral ventricles, the head of the caudate nucleus, the anterior group of thalamic nuclei, the habenular trigone, the superior colliculus, and certain other points more ventrally situated in the lateral wall of the third ventricle, it is hard to see how such experiments could be interpreted as favoring the existence of any closely integrated mechanism regulating the change from the waking to the sleeping state.

There is a remarkable similarity between the behavior of our cataleptic cats and some patients with encephalitis lethargica in which an association of general muscular rigidity with a peculiar lethargy produces a clinical picture closely resembling catalepsy. The ptosis frequently seen in these patients indicates a lesion in the rostral part of the oculomotor nucleus. From pathological studies it is known that the lesions in this disease, while somewhat diffuse in their distribution, are most abundant in the region of transition between the midbrain and diencephalon. On the basis of a study of such cases von Economo (1930) postulated the existence of a center in the region between the mammillary bodies and the third nerve which is responsible for the pathological sleep exhibited by these cases. He conceives of this as a center from which inhibition spreads to the cortex. According to this conception the effective lesion would have to be an irritative one. One might with equal reason think of this as a region which when active radiated excitation to the cortex and that when this excitation was removed lethargy resulted (Pette, 1930). The experiments of Bard (1928) showing that cats, from which all of the brain rostral to the hypothalamus had been removed, were very irritable and exhibited sham rage, might be interpreted so as to lend some support to the latter

hypothesis. But in spite of the widespread interest in this subject during recent years due to epidemics of encephalitis lethargica, accurate information on the subject is far too meager to make such speculation profitable (Kleitman, 1929).

It is, however, generally recognized by clinical neurologists that pathologically prolonged sleep, which except for its duration resembles normal sleep and from which the patient can be readily aroused to clear consciousness only to drop off to sleep again as soon as he is left undisturbed, is a symptom which points to a lesion in the gray matter of the brain stem in the region of transition of the cerebral aqueduct into the third ventricle (Müller, 1931). In encephalitis lethargica the lesions are rather widely scattered through the basal ganglia and brainstem; and although the region of transition between mesencephalon and diencephalon seems to be the region where the lesions are most abundant such cases do not offer very satisfactory evidence as to the localization of the particular lesions responsible for the lethargy. A more satisfactory localization is offered by the case of Pette (1923). A vascular lesion of sudden onset resulted in paralysis of both oculomotor nerves and somnolence, which beginning on the day of onset, persisted until the death of the patient three months later. This sleep differed from coma in that the patient could be readily wakened to clear consciousness but would fall to sleep again as soon as he was left to himself. Autopsy showed an irregular but sharply circumscribed lesion in the tegmentum of the mesencephalon and floor of the third ventricle. In the bibliography are given references to a few of the more important papers which present evidence from clinical neurology to show that lesions located in this region frequently cause somnolence.

SUMMARY

Bilateral electrolytic lesions in the region between the mammillary body and third nerve in cats often lead to a condition of somnolence and exaggerated muscle tonus of a very plastic type. Cats with such lesions offer a striking resemblance to patients with encephalitis lethargica of the cataleptic type. They will maintain for many minutes unusual postures into which they have been placed by the experimenter and the ease with which they can be molded into statuesque postures seems to be directly related to their somnolence and the paucity of voluntary movements.

The lesions in these cats occupy the region of transition between mesencephalon and diencephalon which is known to be involved in certain types of pathologic sleep in man. Since the lesions observed in such cases have never been very sharply localized little is known about the nervous mechanisms involved. The possibility of producing prolonged sleep in cats by placing restricted bilateral lesions rostral to the third nerve offers an opportunity for determining more accurately the location of this mechanism and

for the study of the manner in which it regulates the change from the waking to the sleeping state. Such experiments may also lead to an explanation of why in some patients somnolence is associated with increased plastic tonus, a combination of symptoms known as catalepsy, and why in others it is associated with muscular relaxation.

BIBLIOGRAPHY

BARD, P. 1928. This Journal, lxxxiv, 490.

VON ECONOMO, C. 1930. Journ. Nerv. and Ment. Dis., lxxi, 249.

FULTON, J. F. AND P. BAILEY. 1929. Journ. Nerv. and Ment. Dis., lxix, 1, 145, 261.

HESS, W. R. 1931. Compt. Rend. Soc. d. Biol., cvii, 1333.

HIRSCH, E. 1927. Monatschr. f. Psychiat. u. Neurol., lxiii, 113.

INGRAM, W. R. AND S. W. RANSON. 1932. Arch Neurol. and Psychiat., in press.

KLEITMAN, N. 1929. Physiol. Rev., ix, 624.

MÜLLER, L. R. 1931. Lebensnerven und Lebenstriebe. Chapter by S. REGELSBERGER, 483, Springer, Berlin.

PETTE, H. 1923. Deutsch. Zeitschr. f. Nervenheilkunde, lxxvi, 1.
1930. Klin. Wochenschr., ix, 2329.

PÖTZL, O. 1927. Monatschr. f. Psychiat. u. Neurol., lxiv, 1.

SPIEGEL, E. A. AND C. INABA. 1927. Zeitschr. f. d. gesammt. exp. Med., lv, 164.

THE RATE OF FORMATION OF CEREBROSPINAL FLUID IN ETHERIZED CATS

LOUIS B. FLEXNER AND H. WINTERS

From the Department of Anatomy, Johns Hopkins University

Received for publication June 8, 1932

The literature contains no accurate data on the normal rate of formation of cerebrospinal fluid; as Levinson (1929) has said, this is still a matter of conjecture. Such reports as are at hand have come from observations on the rate of flow of the fluid from fractured portions of the skull and spinal column, or from measurement of the quantity escaping from a cannula placed in the subarachnoid space of an animal. In numerous cases of cerebrospinal rhinorrhea the amount of fluid flowing from the nose has been found to be from 96 cc. to 720 cc. per day (Levinson, 1929). Falkenheim and Naunyn (1887), using dogs and the method of cannulation of the subarachnoid space found a rate of fluid-escape varying from 36 to 240 cc. in 24 hours. Estimates of the normal rate of formation of cerebrospinal fluid from data of this kind have these objections: cerebrospinal fluid pressure is equal to the resistance of the artificial opening and so is far below normal, and no account can be taken of the amount of fluid being absorbed during the period of observation. The interpretation of data from cases of injury to the skull or vertebral column is faced with the additional complications which may come from trauma to the central nervous system and its coverings. These considerations led Weed (1922) to the opinion that estimates of the normal rate of fluid-formation available to him were all probably too high.

METHODS. It has been our aim to devise a method which permits measurement, under normal intraventricular pressure, of the amount of cerebrospinal fluid leaving the aqueduct of Sylvius, during the course of several hours. For this purpose it was necessary to establish and maintain a water-tight block at, or rostral to, the cerebellar peduncles so that fluid escaped neither into the subarachnoid space via the foramina of Luschka nor past the block directly into the region of the cisterna magna. It was further necessary to have an instrument which permitted measurement of changes in fluid-volume occurring at known pressures.

Our first efforts to obtain a water-tight block were made by attempting to catheterize the aqueduct with a small, silk ureteral catheter. This method has, in our hands, been entirely unsuccessful for even after suc-

cessful catheterization leaks were always present between the catheter and the walls of the aqueduct. Our next attempts were made with a small, soft rubber catheter of size 10. This was placed in the fourth ventricle against the opening of the aqueduct and held in position by cotton packs and agar placed rostral to the cerebellar peduncles. To prevent leakage around the catheter a ring of bone wax about 2 cm. high was fixed to the occiput and atlas, and mercury then poured into the ventricle and the bone wax cup. In this way it was possible to maintain a pressure of about 2 cm. of mercury on the cotton packing. This last method has yielded data which we feel to be valid but it has the disadvantages of being both laborious and uncertain.

Most of the data to be reported here have come from measurement of fluid flowing through a catheter placed against the aqueduct of Sylvius and surrounded by a balloon shaped to secure blockage of the ventricle. Glass models of the portion of the fourth ventricle rostral to the cerebellar peduncles were blown from paraffin-wax casts. Rubber balloons were then made over these glass models according to the method of Reynolds and Friedman (1930) which involves use of pure gum caoutchouc dissolved in carbon tetrachloride and vulcanization by sulphur chloride. A small, soft rubber catheter was then introduced into and through the balloon, and, for the purpose of dilating the balloon, a second small catheter was connected. The union of the catheters to the balloon was made by use of thin celloidin. After properly placing the balloon with its catheters in the rostral portion of the ventricle, blockage was accomplished by distending the balloon through the catheter which ended within it. If, then, the fit between balloon and ventricular wall were water-tight, all fluid which escaped from the aqueduct must have flowed from the catheter which penetrated the balloon.

In all these experiments, the amount of fluid leaving the catheter was measured with a bubble-manometer such as used by Weed, Flexner and Clark (1932). This consisted of a long tube of 1 mm. bore with a scale attached so that the distance traversed by a bubble of air in a column of Locke's solution could readily be ascertained. The volume of sections of the tube was measured with mercury and length could therefore be translated into volume. A movable fluid-reservoir of relatively large diameter (15 mm.), attached to one end of the manometer, permitted determination of the rate of cerebrospinal fluid-escape at any desired intraventricular pressure.

It was of course of first importance to be certain that no artifacts entered into the method. There were several possible sources of error. The experiments were made in a room in which no precautions were taken to insure a constant temperature. It was found, however, if care was taken to fill the catheter and its connections with the manometer completely with

Locke's solution and to rid them of all air, that the position of the bubble in the manometer was practically invariable with relatively extreme temperature changes. This factor appeared, therefore, to influence in no appreciable way the correctness of the observations.

More important than this was the demonstration of a water-tight block between the ventricle and the catheter. At the end of each experiment the animal was killed and the apparatus left in position. Drift of the bubble in the manometer toward the animal was then taken as evidence of leakage and the experiment discarded. The test was very sensitive and hence apparently reliable. It led to our discarding most of the results obtained with mercury-blockage and about two out of every three experiments in which balloons were used.

The balloons were distended either with air or water. When water was used, the pressure within the balloon amounted to about 160 mm. of water. It is evident that a small leak in the body of the balloon might have permitted escape of water from the balloon into the ventricle and so produced movement of the bubble in the manometer not due to escape of cerebrospinal fluid. This possibility could be investigated immediately post-mortem with the apparatus in place. In the presence of a leak, the bubble in the manometer instead of being stationary would have moved away from the dead animal. There was the unlikely possibility that a leak of this kind might have been approximately compensated for by a leak between the walls of the balloon and the ventricle and that, therefore, a stationary bubble post-mortem provided a somewhat uncertain test of the correctness of observations made during life. To rule out this slight possibility, some of the balloons were distended with air instead of water. Should air escape from the interior of the balloon into the ventricle and affect the position of the bubble, it must, during the course of an experiment, pass into the catheter and be observable in the glass portions of the apparatus. With the same artifact in mind, the ventricles of animals used in the experiments with mercury were examined post-mortem for any of the metal which might have passed the cotton and agar plug. We have had practically no difficulty from this source of error.

These experiments were all performed on adult cats. The animals were kept at normal body temperature under light surgical ether anesthesia throughout the period of observation. A mid-line incision was made through the skin over the occiput and back of the neck and the muscles attached to the occipital bone and atlas separated and retracted to give exposure of these bones and the occipito-atlantoid ligament. After exposing the cerebellum by rongeuring away a portion of the occipital bone, the dura and arachnoid over the cisterna magna were incised. The cerebellum was then carefully lifted away from the medulla with a spatula and the fourth ventricle entered. After removing the fluid in the ventricle with

cotton pledgets, the aqueduct could readily be seen. The balloon and its catheters were then placed in the rostral portion of the ventricle. Hemorrhage caused no difficulty. At the end of the experiments, all animals were promptly killed.

Throughout these experiments, observations were made with an intraventricular pressure of 110 mm. water ± 10 mm., account having been taken of the resistance of the apparatus. This is a pressure about equal to the average normal as found by Weed and Hughson (1921b) for adult cats.

EXPERIMENTAL DATA. The data from the observations on etherized adult cats may best be presented under several topics:

TABLE 1

EXPERIMENT NUMBER	BODY WEIGHT	BRAIN WEIGHT	CHORIOID PLEXUS WEIGHT	METHOD OF BLOCKAGE	PERIOD OF OBSERVATION	RATE OF C.S.F. FORMATION
	grams	*grams*	*mgm.*		*hours*	*cc. per day*
CV-1				Mercury	2.0	13.4
CV-2				Mercury	2.0	13.2
CV-5				Balloon and H_2O	3.5	8.8
CV-6	2,600	18.0	40	Balloon and H_2O	6.5	15.9
CV-8	2,500	20.0	30	Balloon and H_2O	2.5	15.8
CV-11	3,200	25.6	32	Balloon and H_2O	4.0	10.1
CV-23	3,500	23.5	25	Balloon and H_2O	2.0	10.6
CV-28	2,900	22.0	25	Balloon and air	4.5	11.4
CV-30	3,500	22.5	15	Balloon and air	2.0	13.7
CV-31	3,500	23.0	35	Balloon and air	2.5	13.7
CV-32	3,300	25.6	30	Balloon and air	1.0	9.3
CV-34	3,000	25.0	40	Balloon and air	1.0	12.0
CV-35	2,500	20.5	25	Balloon and air	1.0	10.5
Average						12.1

Rate of flow of cerebrospinal fluid from the aqueduct of Sylvius. Table 1 presents in summary form observations on the rate of flow of cerebrospinal fluid from the aqueduct of Sylvius. On the basis of measurements taken over periods from one to six and one-half hours in 13 adult cats, it has been found that an average of 12.1 cc. of cerebrospinal fluid per day leave the aqueduct. Among individual animals, the quantity appeared to vary between 9 cc. and 16 cc. It is apparent from the table that this variation in rate was not to be correlated with differences of body weight or brain weight. In 10 of the experiments, the chorioid plexuses of lateral and third ventricles were removed, and weighed after surface fluid had been absorbed by filter paper. In all instances, an attempt was made to remove carefully the tela chorioidea from the plexus; nevertheless, a con-

siderable error undoubtedly came from this source. The variations in rate of fluid-escape found no explanation in these weights.

Striking differences in the character of the fluid-stream leaving the aqueduct were noted among the experiments and, on occasion, at different

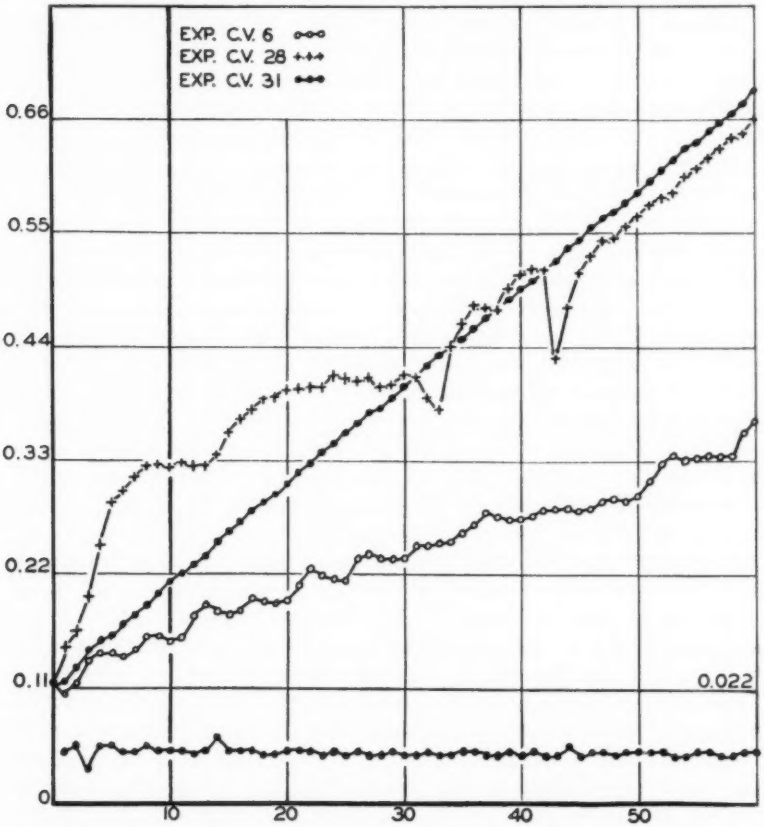


Chart 1 (cats, experiments CV-6, CV-28 and CV-31). The ordinates on the left represent volume in cubic centimeters of cerebrospinal fluid-flow from the aqueduct of Sylvius; the abscissae, time in minutes. The curves portray the three types of flow observed in a series of 13 cats. The ordinates on the right represent volume in cubic centimeters of cerebrospinal fluid-flow and evaluate the lowest curve which expresses rate of flow per minute for experiment CV-31.

periods of the same experiment. Some of the observations demonstrated a rate of flow which was extraordinarily constant, minute after minute, for several hours. In such experiments, exemplified in chart 1 by experi-

ment CV-31, the rate per minute as plotted showed little deviation from a horizontal straight line and the total quantity of fluid which had escaped at any particular time lay on a line of practically constant slope. In this experiment CV-31 almost all of the measurements showed a rate per minute varying only between 0.0088 and 0.0099 cc.

In other animals, exemplified in chart 1 by experiment CV-6, there were long periods in which fluid-flow from the aqueduct occurred in very regular cycles. Periods of active flow were followed by phases in which no fluid left the aqueduct; a rate of about 0.01 cc. per minute suddenly or gradually gave way to a period of two to four minutes in which flow ceased. The whole cycle in experiment CV-6 lasted for an average of about five minutes and the whole period of cyclic flow for about 80 minutes.

Finally, there was a group of experiments in which the rate of flow was highly irregular. As is shown by experiment CV-28 of chart 1, here again there were well defined cycles in which large rates of flow from the aqueduct were followed by periods of cessation. These cycles, however, were very irregular in their times of duration and in their forms; periods of unusually active flow amounting to as much as 0.06 cc. per minute alterated with periods of cessation or intervals during which fluid from the manometer was sucked back into the ventricles. It is to be noted in the graph of this experiment that the period of extreme irregularity was followed by a period in which rate of flow was almost as constant as in experiment CV-31.

Several of the animals showed irregularities of a different sort. Chart 2 shows two such experiments, CV-6 and CV-11, in which the form of the curve and the amount of fluid-escape varied greatly from hour to hour. In CV-6, which showed the most extreme variations, maximum fluid-escape for an hour amounted to 1.5 cc., and minimum, to 0.3 cc. In experiment CV-5 of chart 2, on the other hand, although the curve of fluid-flow was irregular, the amounts of fluid produced per hour were almost identical and showed only slightly greater variation than experiment CV-31 of chart 1. Four of the 13 experiments showed irregular rates from hour to hour and were of much the same type as CV-6 and CV-11; six presented only slight variations in hourly flow though three of these were of the irregular form of experiment CV-5; in three, observations were made for only an hour.

Evidence of ventricular volume changes. Intracranial arterial and venous pressures were recorded simultaneously with rate of cerebrospinal fluid-escape in several of the experiments. Measurements of the pressure in the circle of Willis presented little difficulty; this was accomplished by cannulation of the peripheral end of the carotid artery. Intracranial venous pressure was less readily determined. The superior sagittal sinus in most cats is too narrow to permit puncture with a needle as suggested for dogs by Weed and Hughson (1921a). To measure pressures from

the torcular herophili is often difficult and the method has certain dangers of inaccuracy.

Venous pressures in these experiments have been recorded from the peripheral end of the external jugular vein. This vein in the cat and dog is the largest vein of the neck, the internal jugular being, in all but rare

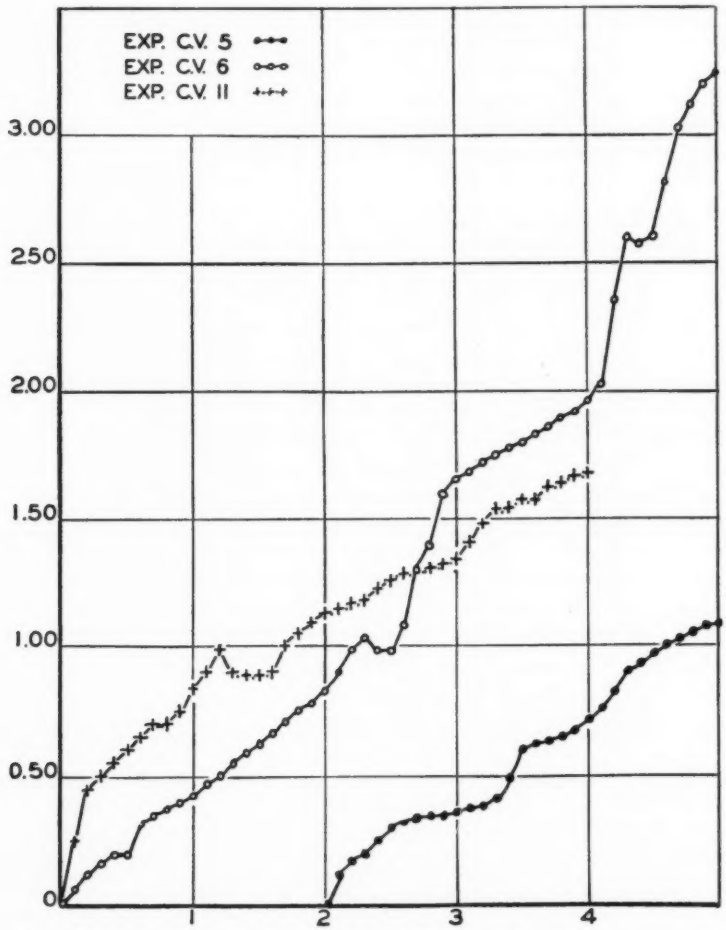


Chart 2 (cats, experiments CV-5, CV-6 and CV-11). The ordinates represent volume in cubic centimeters of cerebrospinal fluid-flow from the aqueduct of Sylvius; the abscissae, time in hours. The curves portray the irregularities of rate of flow over long periods observed in certain experiments (CV-6 and CV-11); and the constancy found in others (CV-5).

instances, of a caliber too small to allow cannulation. Several dissections of the external jugular veins of cats and dogs were made and the relations of these vessels in both animals found to be practically equivalent. In all instances the vein in the neck was found to receive a large branch from within the skull. It was consequently argued that the peripheral end of the vein might be used to record intracranial venous pressure.

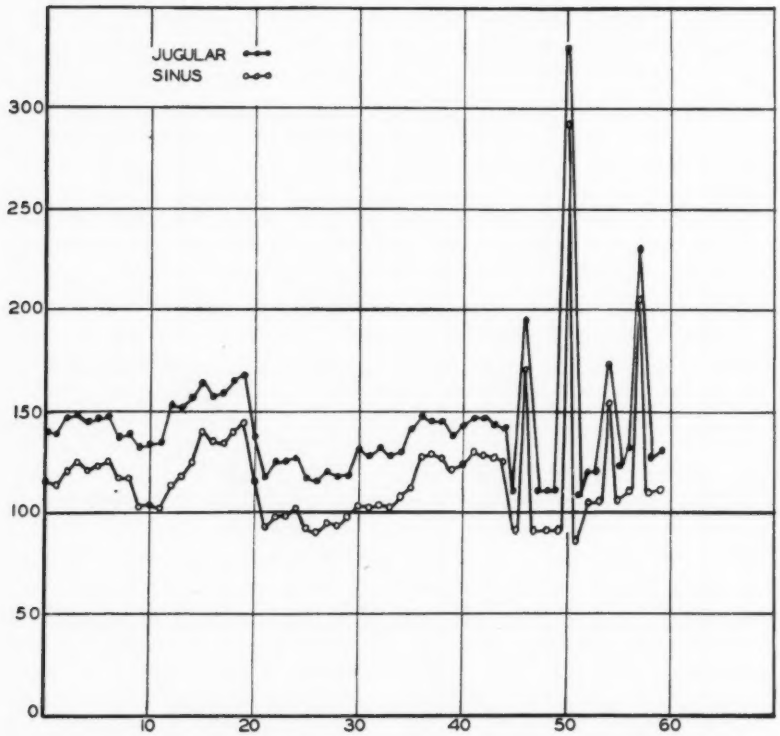


Chart 3 (dog, experiment CV-18). The ordinates represent pressure in millimeters of normal saline solution; the abscissae, time in minutes. The curves show the equality of pressure changes which occurred in the superior sagittal sinus and the peripheral end of an external jugular vein. In the interval from 45 to 65 minutes, intracranial venous pressure was altered by pressing on the intact external jugular vein. Other changes occurred spontaneously.

To put this possibility to test, simultaneous pressure readings were taken from a needle placed in the superior sagittal sinus of a dog according to the method of Weed and Hughson, and from a cannula in the peripheral end of the external jugular vein. Chart 3 presents some of the data from

such an experiment. It is seen that spontaneous pressure changes occurred simultaneously and to almost the same degree in both vessels. Moreover, pressure on the intact external jugular vein produced small or large pressure changes of the same magnitude in the two vessels. The same sort of result followed decrease or increase of intracranial pressure consequent to withdrawal of cerebrospinal fluid from the subarachnoid space or injection of the fluid into it. The pressure in the external jugular vein was always found to be slightly higher than in the superior sagittal sinus; in experiment CV-18 of chart 3 this difference was between 20 and 25 mm. of normal saline, the maximum difference found in three experiments. These observations appeared, consequently, to justify use of the peripheral end of the external jugular vein in the cat and dog for measurement of changes in intracranial venous pressure.

Because of the likelihood of altering the blood supply to structures of the ventricles by cannulation of carotid artery and external jugular vein, arterial and venous pressures were measured in only three of the experiments reported here. Experiment CV-34 of chart 4 presents typically the results of such observations.

It was first necessary to determine the effects on intracranial blood pressures of the necessary manipulations in and about the fourth ventricle. At the time marked by arrow L of the graph (chart 4), the dura and arachnoid over the cisterna magna were incised with escape of cerebrospinal fluid and fall of its pressure to the level of the atmosphere. This procedure was without effect on arterial pressure, but, as was anticipated, caused a decrease in venous pressure amounting in different cases to from 10 to 30 mm. of normal saline solution.

At the time marked by arrow B of the graph (chart 4), the balloon and its catheters were placed in the ventricle and the balloon then distended. In two of the three experiments conducted in this way, distention of the balloon was accompanied by an increase of intracranial arterial pressure amounting to about 10 mm. of mercury. Venous pressure, however, never showed a measurable change. These findings coupled with the finding post-mortem of a fourth ventricle only slightly dilated, led to the conclusion that the pressures used in distending the balloon introduced no important error into the observations.

Changes in intracranial blood volume apparently led to variations in the volume of the ventricles. The bubble of the manometer, with the balloon in place and distended, invariably showed pulsations just as does the fluid meniscus of a manometer connected to the subarachnoid space. Arterial pulsations equivalent to a ventricular volume change of approximately 0.001 cc. and respiratory pulsations amounting to a volume change of approximately 0.01 cc. were frequently noted. These changes were of course of great regularity and influenced in no way the rate of flow per min-

ute from the aqueduct. They demonstrate, however, that distension of the arteries following systole or emptying of the veins with inspiration produce changes in ventricular volume.

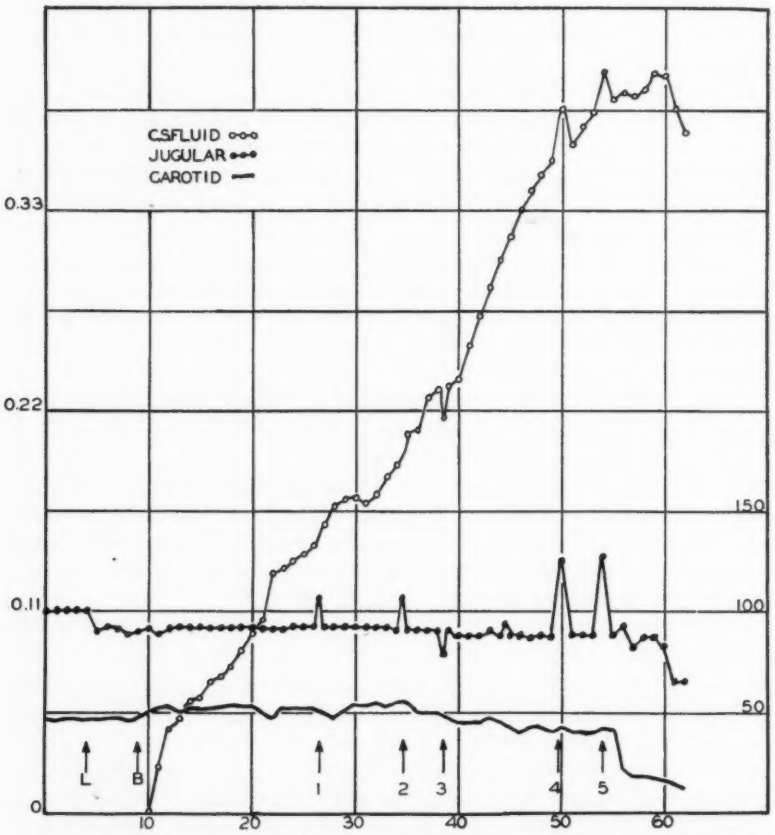


Chart 4 (cat, experiment CV-34). The abscissae represent time in minutes. The ordinates on the left represent volume in cubic centimeters of cerebrospinal fluid-flow from the aqueduct of Sylvius; the ordinates on the right, pressure in millimeters of normal saline solution or mercury (carotid pressure). The curves show the relations found between changes in rate of cerebrospinal fluid-flow and changes in intracranial blood pressures. The significance of the arrows is given in the text.

Changes of the same general sort, not related, in the same way, however, to heart beat or respiration were frequently observed during the course of the experiments. Experiment CV-6 of chart 1, for example, showed

periods as long as three minutes in which fluid was aspirated from the manometer into the ventricle. In other experiments such as CV-28 of chart 1 the change was more striking and as much as 0.1 cc. of fluid was sucked into the ventricle in the course of one minute. On the other hand it has been noted that as much as 0.3 cc. of fluid may, within about 30 seconds, be suddenly expelled from the ventricles. Very often the original position of the bubble was quickly reëstablished but at times there was no recession.

Simultaneous measurement of intracranial arterial and venous pressures and of cerebrospinal fluid-flow from the aqueduct have furnished data which may offer an adequate explanation of these observations. Experiment CV-34 of chart 4 indicated that the changes in arterial pressure likely to occur in the course of a well conducted experiment, with the animal in good condition, did not alter in an appreciable way the rate of flow. Small increases or small decreases in the pressure within the circle of Willis produced no apparent change in rate of cerebrospinal fluid-escape. Rather marked changes in venous pressure occurring without apparent cause or on occasion as a result of movement of the animal were, however, often correlated with changes in the rate of flow. Thus the transitory increases in venous pressure marked by arrows 1 and 2 in chart 4 were accompanied by transitory increases in the rate. Increase in venous pressure from pressure on the intact external jugular vein also caused a momentary increase in flow (arrows 4 and 5 of chart 4). A decrease in venous pressure as marked by arrow 3 caused a decrease in flow. Reëstablishment of venous pressure was accompanied by a resumption of the original rate of flow. It is of interest to note that with approach of the animal's death, fluid in the manometer was sucked into the ventricles coincident with sharp falls in arterial and venous pressures. Obviously, however, marked changes in rate of flow did occur without evidence of change in intracranial vascular pressures. This lack of correlation, very frequently observed, deserves the same emphasis as does the correlation.

Discussion. Three methods, fundamentally alike but differing in important details, have been shown to yield results of the same magnitude for the rate of flow of cerebrospinal fluid from the aqueduct of Sylvius of etherized adult cats. Each experiment was tested as severely as was found possible for artifact and in the event of its demonstration, the experiment was discarded. Two efforts were made to test further the measurements obtained from these methods by plugging the aqueduct with cotton and placing a needle through the corpus callosum and into the third ventricle. These attempts failed because of leakage around the needle.

An average of 12.1 cc. per day of cerebrospinal fluid has been found to flow from the aqueduct of the adult cat under ether anesthesia. Whether or not this is to be taken as an accurate statement of the rate of formation

in the normal cat is at the moment uncertain. Analysis of this possibility awaits investigation of the effects of ether on the rate of formation of the fluid.

It would of course be of much additional value if an average could be given for the amount of fluid which leaves the four ventricles to flow into the subarachnoid space. Technical difficulties have made direct measurement of this quantity impossible. It has been found, however, that the chorioid plexus of the fourth ventricle weighs on the average about 25 per cent as much as the plexuses of the other three ventricles. If the assumption be made, and this may well be entirely unjustifiable, that the amount of fluid formed from a single plexus of any individual animal is approximately a direct function of the plexus weight, then an addition of 3 cc. per day must be made. This would give an average outflow of cerebrospinal fluid from the ventricles of 15 cc. per day.

The average value given here has come from experiments lasting from one to six and one-half hours, and averaging a little more than two and one-half hours in duration. It can be urged that these intervals are too short to permit deductions as to the amount of cerebrospinal fluid formed over a period of 24 hours. Rate of formation of cerebrospinal fluid may well show large fluctuations in the same individual as has been suggested by Hart (1927) and has been demonstrated in some of the experiments reported here. A considerable error may consequently appear to have been introduced in the calculations for a day. The only answer which can be made to this criticism is that a rather large series of animals have been studied and that they have all given results of the same magnitude. It is likely that they presented various degrees of activity of fluid-formation and that, therefore, the average figure presented affords a fair estimate of the rate per day in the cat.

It has been pointed out that there are apparently three distinct modes of flow, varying from almost perfect regularity through regular cycles of changing flow to a type of great irregularity. No particular type is to be taken as characteristic of an individual animal. It appears probable that in all animals, over a long period of time, the character of the fluid-stream leaving the aqueduct changes in these three possible ways.

Such changes as these must find their explanation in two causes: first, actual variations in the rate of formation of cerebrospinal fluid and second, variations in the volume of the ventricles. That changes in ventricular volume do occur as the result of variations in the blood volume of their walls, appears to be substantiated by considerable evidence. Throughout these experiments, rise in venous pressure has been taken as an index of increased distention of the veins and hence an increase in venous blood volume; and fall in venous pressure, as evidence of decrease in venous blood volume. It has been shown that a decrease in venous pressure was ac-

companied by aspiration of small amounts of fluid into the ventricles and that increased venous pressure apparently caused expulsion of fluid from the ventricles. These changes, as far as they have been studied, have been characterized by small reaction times, the maximum noted being five minutes, most occurring in less than a minute. The ventricles, therefore, apparently are a part of the extensive mechanism of reciprocal compensation within the bony coverings of the central venous system. They share in the reactions which keep total volume of cerebrospinal fluid, blood and central nervous system practically constant as is demanded by the Monro-Kellie doctrine.[1]

There are, however, changes in rate of flow which are not accompanied by measured changes in blood pressure. These are frequently different from those just mentioned in that they last for long periods of time and appear due to actual variations in the rate of formation of cerebrospinal fluid. An explanation for these variations is entirely problematical. To argue that they are independent of arterial pressure is misleading for the pressure as measured in the circle of Willis is but a crude indication of pressure-changes taking place within the capillaries of the chorioid plexus; and knowledge of this quantity can alone provide an unequivocal analysis. In the same way, measurement of venous pressure gives only a view of changes occurring within the large sinuses of the head and tells little of small but probably important changes in the venules and veins of the structures of the ventricles.

Care has been taken throughout this report to speak of rate of flow or escape of cerebrospinal fluid, rather than its rate of formation. This has been done because the description of results was largely concerned with changes taking place over short periods of time. These changes, as has been discussed, appear related not only to actual rate of formation but to ventricular volume-changes as well. In evaluating measurements made over a long period, however, it appears wholly justifiable to speak of rate of formation of cerebrospinal fluid. Ventricular volume changes, over these periods, become inconsequential.

It is of interest to inquire how nearly the amount of cerebrospinal fluid formed within the ventricles approaches the total quantity produced by the animal. An analysis of this problem involves a quantitative knowledge of the amounts of fluid escaping from the perivascular spaces and from the blood vessels of the subarachnoid space. Exact information in this regard is lacking and any statement in consequence is conjectural. The work of Weed (1922) and Schaltenbrand and Bailey (1928), however, indicates that normally the perivascular spaces contribute a very small quantity of fluid to the subarachnoid space. Nor is there any good evidence that

[1] For a general discussion of reciprocal compensation, see L. B. Flexner, J. H. Clark, and L. H. Weed, This Journal, 1932, ci, 292.

cerebrospinal fluid leaves the vessels of the subarachnoid space. With our present knowledge, therefore, it is perhaps justifiable to conclude that measurement of the amount of cerebrospinal fluid leaving the ventricles gives a quite accurate estimate of the total quantity of fluid produced. This conclusion is only tentative, however, and with further investigation, may well prove to be untenable.

SUMMARY

Using three methods, fundamentally alike but differing in important details, the average rate of flow of cerebrospinal fluid from the aqueduct of Sylvius in the etherized adult cat has been found to be 12 cc. per day. The rate in a series of 13 cats varied between about 9 cc. and 16 cc. of fluid per day. These differences found no explanation in weight of body, brain or chorioid plexuses.

Three distinct types of flow were found among the animals. Changes in pressure within the circle of Willis afforded no explanation of these findings. Some of the deviations from a constant rate of flow, however, were correlated with intracranial venous pressure-changes and are considered to be evidences of ventricular volume change.

BIBLIOGRAPHY

FALKENHEIM, H. AND B. NAUNYN. 1887. Arch. f. exper. Path. u. Pharm., xii, 261.

HART, D. 1927. Arch. Surg., xv, 943.

LEVINSON, A. 1929. The cerebrospinal fluid. 3rd ed., St. Louis.

REYNOLDS, S. R. M. AND M. H. FRIEDMAN. 1930. This Journal, xciv, 696.

SCHALTENBRAND, G. AND P. BAILEY. 1928. Journ. f. Psychol. u. Neurol., xxxv, 199.

WEED, L. H. 1922. Physiol. Reviews, ii, 171.

WEED, L. H. AND W. HUGHSON. 1921a. This Journal, lviii, 101.

1921b. This Journal, lviii, 53.

WEED, L. H., L. B. FLEXNER, AND J. H. CLARK. 1932. This Journal, c, 246.

EFFECT OF POSTERIOR PITUITARY EXTRACTS ON THE CONSTITUENTS OF THE BLOOD

H. E. HIMWICH, F. W. HAYNES AND J. F. FAZIKAS

From the Department of Physiology, School of Medicine, Yale University, New Haven, Connecticut

Received for publication June 10, 1932

The effects of the posterior pituitary extract on blood constituents have been extensively studied in recent years. The rise in blood sugar obtained with pituitrin (Partos and Klatz-Klein, 1921; Burn, 1928; Clark, 1925; Tingle and Imrie, 1926) is effected also by pitocin and pitressin (Geiling, 1932; Nitzescu and Benetato, 1930; Bacq and Dworkin, 1930). Other experiments (Himwich and Fazikas, 1930; Bischoff and Long, 1931) have shown that pitressin and pituitrin also cause a rise in blood lactic acid. Since it has been found that the respiratory metabolism is affected differently by the three pituitary extracts (Himwich and Haynes, 1931) a differential study of their effects on the lactic acid and glucose content of the blood of unanesthetized and amytalized animals has been made in an attempt to explain the various changes produced in metabolic rate. The concentration of the blood as a criterion of blood dilution was measured since an increase in blood volume after pituitrin has been observed (Konschegg and Shuster, 1915; Underhill and Pack, 1923).

METHOD. Chloretone-free pituitrin, pitressin, and pitocin were injected subcutaneously into amytalized and unanesthetized dogs in doses of 1.0 to 4.6 pressor or oxytocic units[1] per kilo. These doses were not lethal and the dogs were in good condition at the end of the experiment. Injections were made every 15 or 30 minutes and blood samples of 5 to 15 cc. were drawn from the femoral artery, usually at intervals of two hours. Analyses were made for lactic acid by the method of Friedemann, Cotonio, and Shaffer (1927) and for sugar by the method of Hagedorn and Jensen (1923). Total solids were determined from the dry weight of 1 cc. of plasma. In order to determine the effect on the plasma concentration of merely drawing blood control samples were taken from unanesthetized animals. Because of the irregularity of the results under amytal anesthesia, 2 dogs were decerebrated before study.

[1] One cubic centimeter pituitrin contains 10 oxytocic and 10 pressor units. One cubic centimeter pitocin contains 10 oxytocic units and 1 cc. pitressin contains 10 pressor units. These substances were generously supplied by Parke, Davis & Company.

RESULTS. The results of this series of experiments are summarized in table 1. A plus indicates a rise, a zero no change, and a minus a fall in the blood constituent. The figures show the number of experiments in which such changes occurred. Changes greater than the experimental error (at the foot of each column) are considered significant.

It may be seen that pitressin and pitocin as well as pituitrin raised the blood glucose in unanesthetized animals. The average increase in milligrams per cent was 57 for pituitrin, 29 for pitressin and 17 for pitocin. The rise in sugar was less consistent in the case of amytalized dogs and was even reversed after pitocin and pitressin. Chloralose, however, did

TABLE 1

Changes in blood constituents after pituitary extracts

	BLOOD GLUCOSE			LACTIC ACID			TOTAL SOLIDS OF PLASMA		
	+	0	−	+	0	−	+	0	−
Pituitrin unanesthetized	3	0	0	2	2	0	0	0	6
Pituitrin amytalized	3	0	1	0	2	1	0	0	1
Pitocin unanesthetized	3	0	0	0	5	1	0	1	5
Pitocin amytalized	0	0	3	0	1	0	2	0	2
Pitressin unanesthetized	4	0	0	5	0	0	1	0	4
Pitressin amytalized	1	0	3	2	1	0	2	0	4
Control, unanesthetized							0	0	4
Experimental error	±2.5 mgm. %			±2.5 mgm. %			±3 mgm. cc.		
Remarks							In 2 of the controls the fall was preceded by a slight rise		

not have the same effect as amytal since injections of pitressin produced a great rise in the concentration of blood sugar in each of 3 observations.

Determinations of lactic acid in unanesthetized dogs reveal that pitressin caused a rise of 9 to 27 mgm. per cent whereas pitocin had little effect. A few experiments in which tissue lactates were determined have failed to show definite results.

The change in total solids after pitressin and pitocin indicated a dilution of the blood of unanesthetized animals similar to the dilution found after pituitrin. The effect may be partly due to merely drawing blood.

Experiments on decerebrate dogs are difficult to evaluate since after an initial rise blood glucose and lactic acid decrease (Himwich, Koskoff and Nahum, 1930). In spite of this fact pitressin caused a rise in lactic acid in both experiments on decerebrate animals.

DISCUSSION. Himwich and Haynes (1930) found that pitressin decreased oxygen consumption of unanesthetized rats. Recently Geiling (1932) noted that when pitressin was administered the venous blood returned in an arterialized condition indicating that the tissues with the exception of the brain were removing little oxygen from the blood. As a result of diminished oxidations it might be expected that the liver glycogen would break down to glucose and so appear in the blood and that muscle glycogen would undergo cleavage to lactic acid which would also be found in the blood. This would appear to be the case as a rise in the lactic acid of the blood plasma is caused by pitressin but not by pitocin which does not depress the oxygen consumption. Pituitrin lowers the metabolic rate to some extent and probably increases the blood lactic acid.

Experiments with posterior pituitary extract in general may throw some light on the action of pitressin, its pressor fraction. A diminution of liver glycogen after pituitary extract has been found (Burn and Ling, 1929), suggesting a greater output of glucose or a decreased formation of glycogen from lactic acid. Other workers (Lawrence and McCance, 1931; Bischoff and Long, 1931) have failed to confirm these results since they could find no definite change in muscle or liver glycogen after pituitrin. The latter observers also suggest an increased removal of sugar by the peripheral tissues which makes explanation even more difficult.

Raab (1926) has demonstrated that the effect of pituitrin on blood fat is mediated by centers in the diencephalon. It is known that "Dial" acts on the hypothalamus and medulla (Fulton, Liddell and Rioch, 1930) and it is probable that amytal decreases the sensitivity of these centers on which pituitrin may act. In a similar manner avertin anesthesia arrests the action of pituitrin injected into the brain ventricles (Cushing, 1931). The dosage which is effective in unanesthetized dogs may be without effect on animals under amytal. Thus it is seen in the present experiments that with all three extracts the blood sugar and lactic acid often remained unchanged or were lowered in anesthetized animals. We cannot say, however, that the only action of posterior pituitary extract is its direct action on the hypothalamus (in which lie the chief centers of the autonomic nervous system) since pituitrin and pitressin also decrease the oxygen intake of excised tissues (Himwich, Finkelstein and Humphreys, 1931) which are removed from nervous control.

SUMMARY

Observations of the effects of pitressin, pitocin and pituitrin on plasma glucose, lactic acid and total solids were made on 17 amytalized, 21 unanesthetized and 2 decerebrate dogs.

Pitressin, pitocin and pituitrin increase the blood glucose in unanesthetized animals but often lower it in amytalized animals.

Pitressin increases plasma lactic acid in unanesthetized dogs whereas pitocin has little effect.

Pitressin and pitocin probably cause a dilution of the blood similar to that observed after pituitrin.

We wish to thank Miss Marjorie H. Hurlburt for aid in preparation of the manuscript.

The expenses of the research were met in part by a grant-in-aid from the Research Fund of the Yale University School of Medicine.

We wish to thank Parke, Davis & Co. and Dr. Oliver Kamm for a generous supply of the posterior pituitary extracts.

BIBLIOGRAPHY

Bacq, Z. M. and S. Dworkin. 1930. This Journal, xcv, 605.
Bischoff, F. and M. L. Long 1931. This Journal, xcvii, 215; xcix, 253.
Burn, J. H. 1928. Quart. Journ. Pharm., i, 509.
Burn, J. H. and H. W. Ling. 1929. Quart. Journ. Pharm., ii, 1.
Clark, J. A. 1925. Journ. Physiol., lix, 466.
Cushing, H. 1931. Proc. Nat. Acad. Sciences, xvii, 248.
Friedemann, T. E., M. Cotonio and P. A. Shaffer. 1927. Journ. Biol. Chem., lxxiii, 335.
Fulton, J. F., E. G. T. Liddell and D. McK. Rioch. 1930. Journ. Pharm. Exper. Therap., xl, 423.
Geiling, E. M. K. 1932. Johns Hopkins Hosp. Bull., li, no. 1.
Hagedorn, H. E. and B. N. Jensen. 1923. Biochem. Zeitschr., cxxxv, 46.
Himwich, H. E. and J. Fazikas. 1930. Proc. Soc. Exp. Biol. and Med., xxviii, 331.
Himwich, H. E., R. Finkelstein and K. E. Humphreys. 1931. Proc. Soc. Exp. Biol. and Med., xxix, 233.
Himwich, H. E. and F. W. Haynes. 1931. This Journal, xcvi, 640.
Himwich, H. E., Y. D. Koskoff and L. H. Nahum. 1930. Journ. Biol. Chem., lxxxv, 571.
von Konschegg, A. and E. Shuster. 1915. Deutsch. Med. Wochenschr., li, 1091.
Lawrence, R. D. and R. A. McCance. 1931. Biochem. Journ., xxv, 570.
Nitzescu, I. and G. Benetato. 1930. Compt. rend. Soc. Biol., ciii, 1359.
Partos, A. and F. Klatz-Klein. 1921. Zeitschr. f. d. gesammt. Exp. Med., xxv, 99.
Raab, W. 1926. Zeitschr. f. d. gesammt. exper. Med., xlix, 179.
Tingle, C. D. and C. B. Imrie. 1926. Journ. Physiol., lxii, 2.
Underhill, F. P. and G. T. Pack. 1923. This Journal, lxvi, 520.

The American Journal of Physiology is issued monthly by the American Physiological Society under the direction of the Council of the Society. From three to four volumes, each of about seven hundred and twenty pages are published yearly. The subscription price per volume in the United States and Canada is $7.50; in other countries, $8.00.

Manuscripts submitted for publication may be sent to any member of the Council or to the Managing Editor, Dr. D. R. Hooker, 19 W. Chase Street, Baltimore, Md. They should be packed flat, carefully protected from injury, and insured.

Each article should conclude with a brief summary of results suited to the needs of abstract journals.

References to literature should accord with the following arrangement. In the text author's name is to be followed by the year date of the publication in parenthesis, thus Smith (1911). If the author's name is not mentioned in the text, the parenthesis is to contain that name as well as the date, thus (Smith, 1911). If reference is made to more than one publication of an author in a given year, the year date is to be followed by the letters a, b, c, etc., thus Smith (1911a). The bibliography at the end of the article is to list all text references according to authors' names, with initials, alphabetically arranged, thus, Smith, J. C. 1911. Journ. Biol. Chem., xii, 306.

Authors are especially urged to verify personally the accuracy of their bibliographies by reference to the original sources, since inadvertent errors cause readers great inconvenience.

All figures for illustration must be submitted in such form as to admit of photographic reproduction without retouching or redrawing. Marginal letters or figures cannot be set in type and must, therefore, be drawn in India ink by the author and regarded as part of the figure. Unless specific instructions are given by authors, the printer will be allowed to use his judgment in determining the amount of reduction on all figures. The JOURNAL cannot accept illustrations which, when reproduced for printing, exceed the size of a JOURNAL page ($4\frac{1}{2} \times 7\frac{1}{2}$ inches), unless the author is prepared to pay the cost of an insert page.

Plotted curves must be drawn in India ink on **blue lined coördinated paper.** Blue ink will not show in reproduction, so that all guide lines, letters, etc., must be in India ink.

Best results are obtained when such figures are made proportional to the shape of the JOURNAL page so that, when reduced, they will fit without undue loss of space.